THE CHICAGO MEDICAL SCHOOL
QUARTERLY
VOLUME 20 MAY 1959 NUMBER 2

CURRENT CONCEPTS OF PARENTERAL FAT NUTRITION*

GERALD H. BECKER, M.D.**

For a number of years there has been growing recognition of the need for high caloric nutrition in many medical and surgical conditions which are encountered with considerable frequency. Specifically, the problems are those in patients who have lost considerable weight, who cannot or should not consume food by mouth, and whose nutritional status must be maintained or improved as part of the over-all program necessary for a successful convalescence.[1-9]

Convalescence can be viewed as a dynamically evolving process during which the human organism attempts to strike a *positive balance* between anabolic activity on the one hand, and catabolic activity on the other. This basic premise pertains whether one is dealing with post-operative repair or convalescence from operative surgery or convalescence from a major medical illness. The *efficiency* with which this balance can be brought about depends, of course, upon many factors, but primarily the following: 1) the duration of the illness, 2) body reserves, 3) the degree of stress or trauma, 4) certain hormonal factors (mainly adrenal cortex), and last, but of greatest importance, the availability of the raw material, *i.e.*, the utilizable calories which must be present for repair to be accomplished.

In clinical conditions such as chronic ulcerative colitis, intestinal obstruction, renal failure, sprue, chronic diarrhea, complicated duodenal ulcers, and post-operative gastrointestinal surgery, the heart of the problem is caloric deficiency.

The theoretical and practical advantages which intravenous alimentation offers in supplying these calories needs no elaboration. However, all of the intravenous solutions now in general use share a common inability to provide sufficient calories in a reasonable fluid volume. Of the available parenteral fluid preparations, 5% glucose for example provides only 200 cal/liter protein hydrolysate the same; and even if 10% solutions are utilized, only 400 calories per liter are realized. Intravenous alcohol, if completely metabolized, may provide 7 calories/gram, but here again evidence is lacking regarding complete utilization of this substance. In addition, the central nervous system and cardiovascular side effects of alcohol given intravenously are difficult to anticipate and to control. All of the preparations previ-

* The investigations reported in this paper were supported by a research grant from the Research and Development Division, Office of the Surgeon General, Department of the Army.

** Clinical Assistant Professor of Medicine, The Chicago Medical School; Adjunct Attending Physician, Department of Medicine, Michael Reese Hospital.

The Michael Reese Research group consists of the following people: Dr. Fred Adler, Dr. Henry Rosen, Dr. Garold Gussack, Dr. Phillip Levy, Dr. Abraham Gimble, and Dr. Melvin Buxbaum, with the technical assistance of Mrs. Louise Bell.

We are particularly indebted to Lt. Col. William Cox, of the Surgeon General's office, and to Dr. Rachmiel Levine for their co-operation.

ously mentioned except whole blood are excreted by the kidney in varying amounts, and hence the theoretical calories are not always available. Slow rates of infusion do tend to minimize losses via the kidney, but long periods of infusion are difficult for the patient. It is therefore apparent that it is somewhat unusual to be able to supply a patient with much more than 1000 cal/day by means of calorie supplements available unless one is willing to employ rather large volumes of fluid. In many patients who require such intensive therapy, the coexistence of cardiovascular or renal complications may in fact dictate the use of *small* volumes of fluid.

Patients with the conditions mentioned above may need a considerably higher caloric intake than 1000-1200 calories. What the basal caloric needs in a seriously ill individual are is still somewhat controversial. Most observers agree that they may be considerably more than the 1500 calories required by the average well adult and surely if weight loss has been profound, a daily intake of twice the basal requirements would no doubt be beneficial.

It has been said that for protein to be utilized effectively for anabolic purposes, the basal caloric needs of the body must be met from sources other than protein.[10-13] There is ample evidence which indicates that if a high enough caloric intake can be achieved, even in the presence of a bare minimal protein intake, protein can be spared for tissue synthesis. Specifically, a normal male on a nitrogen free diet will exhibit maximal reduction in nitrogen deficit if given a total intake of 750 non-protein calories. Even if this caloric intake is doubled without adding protein, a negative nitrogen balance will still exist. However, when the basal protein requirement of 0.5 gm. 1 kgm is added, there is noted a prompt reversion toward a positive nitrogen balance. Thus, while the need for calories in the nutritionally depleted patient is compelling, there is a *qualitative* aspect to this requirement which is of utmost importance.

The use of fat in the form of a stable emulsion is admirably suited for the purpose of providing these much needed calories on the following basis:

1) It provides 9 calories/gram instead of 4 provided by glucose or protein.
2) It is not excreted by the kidney nor in the stool, and thus all calories given are available for metabolic use.
3) It is non-irritating to the vein as hypertonic glucose may be. In the past fifteen years an impressive body of literature has been published clearly establishing the fact that fat administered parenterally is readily available as a source of energy for metabolic use.

The idea of using fat intravenously is not new. The parenteral administration of milk with cod liver oil was attempted by Whittaker in 1876. Numerous other attempts were made during the early decades of this century with unimpressive results. In 1943 Stare and his group at Harvard, using soybean lecithin as a stabilizer and cottonseed oil as the main constituent, developed the first relatively satisfactory emulsion for clinical use.

In the latter part of 1951, the Office of the Surgeon General became actively interested in this entire problem, and established *The SGO Task Group for the Study of Parenteral Fat Nutrition**. Through their auspices and with the cooperation of the Upjohn Company, a new and less toxic emulsion than previously used by the Harvard group was prepared**.

This preparation is a 15% cottonseed oil emulsion which is prepared by high pressure homogenation. The components of this preparation are listed in table 1, together with the caloric value. The constituent oil is held in a very fine physical emulsion in which all of the particles are 0.5 to 1 micron in diameter as determined by dark field microscopy.

---

* Members of this group are the Harvard School of Public Health, Vanderbilt U. Medical School, Michael Reese Hospital, Walter Reed Hospital, U. of California Medical School, and Louisiana State Medical School.

** Lipomul-IV-Upjohn.

TABLE 1. —*Composition of Lipomul-I.V.*

	Per 100 ml.	
	Gm.	Cal.
Cottonseed oil (special)	15	135
Phosphatides (purified soya lecithin)	1.2	9
Pluronic F68	0.3	0
Dextrose	4.0	16
Water for injection	q.s.	
Total		160

Extensive tests have indicated that this material is biologically and physically stable for at least one year when stored in the refrigerator. Vigorous shaking for 72 hours at 5° C., has resulted in no change in particle size.

Method of Administration

The emulsion is administered through a standard intravenous plastic tubing set using a 20 or 21 gauge needle. Ordinarily a Y tube apparatus is used as with a blood transfusion with the initial infusion being a bottle of 5% glucose in water. Once the system is regulated, the fat emulsion bottle is opened. This is done because occasionally a *patient will experience a flush reaction with the first few milliliters* of the infused fat. Since this is a transient phenomenon, merely clamping the tube to the emulsion and switching to the glucose for a few minutes will permit the infusion of fat to be continued without further incident. It has been accepted procedure *never* to infuse fat through tubing which has previously contained saline for there is a possibility of some disruption of the emulsion under such circumstances.

The infusion is given at a rate of 2 ml. 1 minute for the first 30 minutes and if during this period there has been no indication of any of the clinical reactions which will be discussed below, the rate is increased to 4 ml./minute. An entire 500 milliliter infusion is thus administered over a 3 hour period, with approximately 25 grams of fat per hour given during this period. Figure 1 demonstrates that under these conditions, the rate of removal of the infused fat as judged by total lipid analysis is fairly constant. The maximal fat concentration is reached at the end of the infusion, and 4 hours later the lipid level is still approximately 20% above the control fasting level. The dotted line indicates that when a slower rate of 2 ml./minute is used, the resultant curve is flat and indicates that the infused fat is being removed from the blood at essentially the same rate of administration.

Clinical Reactions

Numerous reactions have been reported by various observers since the initial use of parenteral fat many years ago. These have ranged from nausea to complete cardiovascular collapse. With the current cottonseed emulsion, the combined clinical experience of the various members of the SGO Task Group has now exceeded 5000 separate infusions. During the course of this experience, the following clinical reactions have been recognized:

A. *Major Reactions*

1. Fever — 7% incidence
2. Chill — 1.3% incidence
3. Immediate or Colloid Reaction — less than 1% incidence

This reaction occurs in the first few minutes of infusion and is marked by a flush sensation, dyspnea, urticaria, bradycardia, transient fall in blood pressure, and back pain. This symptom complex usually subsides spontaneously as soon as the infusion of fat is interrupted, and ordinarily does not recur if the infusion is resumed after a 15-20 minute interval. The cause of this response is not known,

but it has been thought to be of vagal origin.

B. *Minor Reactions* — 4% incidence
   1. Headache
   2. Abdominal fullness
   3. Dizziness
   4. Minor variation in pulse and blood pressure

From a practical point of view, the occurrence of fever has been for many years one of the major obstacles to the clinical use of fat emulsions. By definition, any elevation of 2° F. above the control rectal temperature is considered a febrile reaction. Two distinct types of febrile reactions have been observed.

1. The immediate type occurs within 30 to 60 minutes after the start of the infusion and almost invariably is accompanied by a chill. This is probably due to pyrogens in the emulsion or the emulsifying sys-

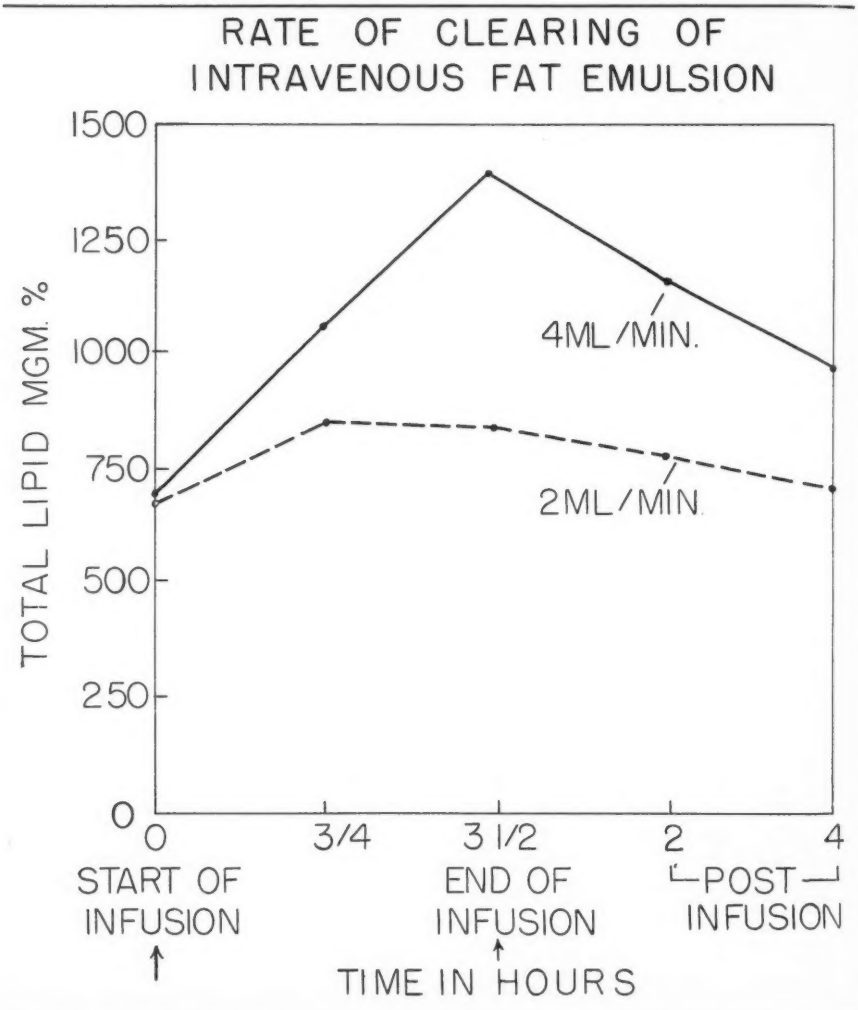


FIGURE 1

tem and has been largely eliminated by recent technical refinements in the preparation of the emulsion.

2. The delayed type is by far the most common of the febrile reactions. The onset is usually 1 to 4 hours after the end of the infusion, and the elevation may be as much as 3-4° F. A chill is not invariably present, and the duration of the fever is usually 2 to 6 hours. The actual cause of this febrile response is not known. Previously it has been attributed to hemolysis, to impurities in the oil, or to pulmonary emboli. However, none of these explanations have ever been supported experimentally. Some observers have indicated that the fever is metabolically induced in the sense that it is due to heat production arising from rapid oxidation of the infused fat which is

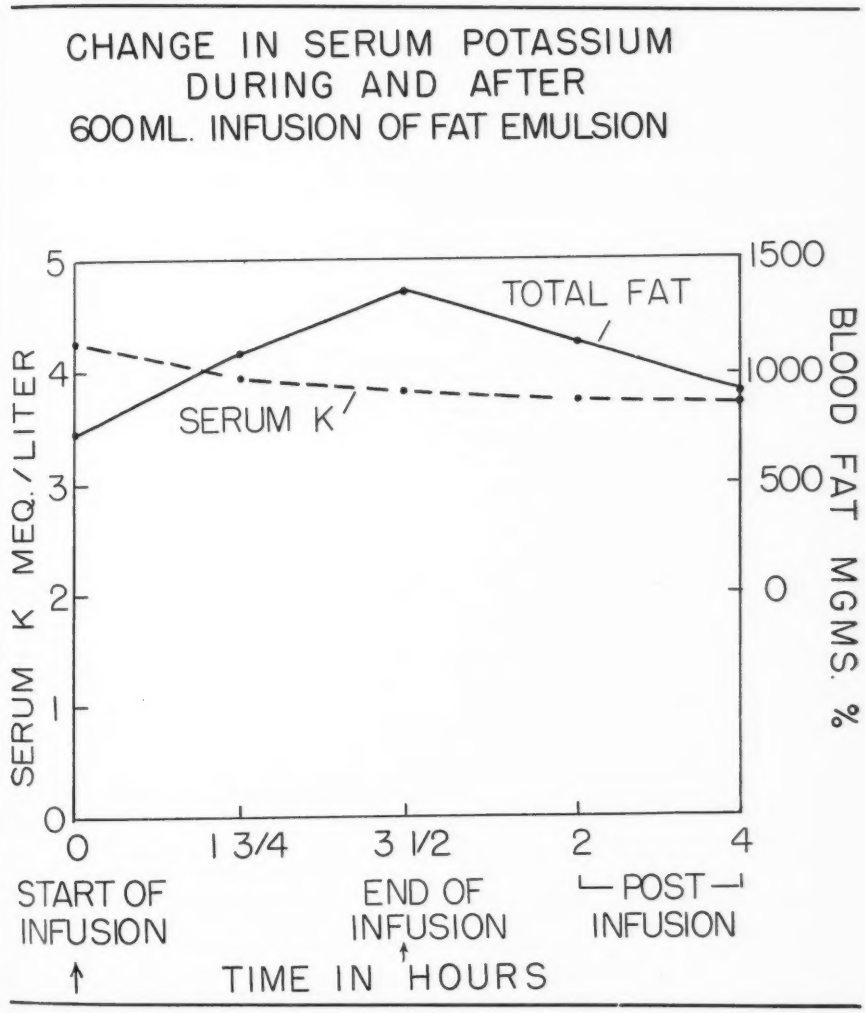


FIGURE 2

available for immediate metabolism. While this theory lacks proof, it may have merit, for it is precisely those patients who clear the infused fat most rapidly who are apt to develop febrile reactions.[14-26].

Electrolyte Studies Following Fat Infusion

Despite a rather extensive experience with various intravenous fat preparations over the past decade, relatively little information had been available on changes which may occur in the electrolyte status of patients receiving such infusions. Since most of these patients are critically ill and may have preexistant electrolyte derangements, information of any changes which would be apt to occur would be of value to the clinician. Furthermore, patients with acute or chronic renal failure are considered ideal candidates for parenteral fat therapy since they require a maximal caloric intake to prevent endogenuos protein breakdown in as small a fluid volume as possible to avoid any cardiovascular complications. These patients already have a metabolic acidosis and therefore, it seemed imperative to us to determine whether the infusion of fat would in any way alter this state.

In the past two years this problem has been under investigation by our laboratory. The effects of single, 500 ml. infusions on plasma electrolyte levels in 25 normal subjects are shown in table 2. It may be noted that with the exception of potassium, where a mean maximal decrease of 8.9 percent was seen, no significant changes occurred in any of the electrolytes measured. The fall in plasma potassium was maximal between the end of the infusion and 2 hours post infusion at a time when the total fat concentration is at a peak level. (Fig. 2)

Table 3 demonstrates that as in the normal group, no major changes occurred in the electrolyte studies conducted on a group of 96 abnormal* patients. However, a tendency for a fall in plasma potassium to occur is again noted, the mean maximal decrease being 9.6%. Again, the major decline occurred between the end of the infusion and 2 hours post infusion. Of interest was the fact that in the abnormal group the magnitude of the individual decrease in potassium was more marked than in the normal subject, the range being -4 to -35 per cent.

All values were corrected for per cent of serum water content; therefore, the change in potassium is not a dilution effect. The precise mechanism of this change is not known, although it seems likely that the combination of fat and glucose in the emulsion acts as an effective medium for intracellular transport

TABLE 2 —*Plasma Lipid and Electrolyte Values* in 25 Normal Patients Receiving 500 ML. of Fat*

Time	During infusion		End of infusion (3½ hr.)	After infusion		Mean maximal change (%)
	0	after 1½ hr.		2 hr.	4 hr.	
Total lipid (mg. %)	744	1092	1478	1191	1053	—
Plasma Na (mEq./L.)	142.3	137.9	136.9	141.5	142.2	−3.8
pH	7.43	7.41	7.41	7.43	7.40	−0.4
Plasma K (mEq./L.)	4.29	4.15	4.02	3.91	4.08	−8.9
Plasma Cl (mEq./L.)	103	101.8	101.8	102.8	103.1	−1.2
CO_2† (mEq./L.)	20.4	18.8	20.6	19.6	22.0	−7.8† +7.8

* All values given are mean values for the group.

† Six results were elevated; five results were decreased.

* The abnormal subjects were all cachectic and were being treated for chronic illness, mainly some form of malignancy.

TABLE 3 — *Plasma Lipid and Electrolite Values in 96 Abnormal Patients Receiving Single 500 ML. Infusions of Fat.*

Time	During infusion		End of infusion (3¼ hrs.)	After infusion		Mean maximal change (%)
	0	After 1¾ hrs.		2 hrs.	4 hrs.	
Total lipid mgm. per cent.....	686	1057	1351	1118	949	
Plasma Na mEq./L.....	138.9	135.7	135.3	136.3	138.3	−2.6
S. D.....	±(4.94)	±(5.03)	±(5.24)	±(5.89)	±(4.20)	
pH.....	7.5	7.43	7.43	7.45	7.48	−0.5
S. D.....	±(1.10)	±(0.22)	±(0.24)	±(0.24)	±(0.22)	
Plasma K mEq./L.....	4.2	4.03	3.89	3.86	3.9	−9.6
S. D.....	±(0.95)	±(0.96)	±(0.96)	±(0.86)	±(0.84)	
Plasma Cl MEq./L.....	99.2	98.4	98.3	98.6	98.5	−0.9
S. D.....	±(4.78)	±(4.73)	±(4.21)	±(5.08)	±(4.89)	
CO_2 mEq./L...	22.5	21.6	20.8	21.4	22.4	−7.6
Serum water (%).....	91.7	91.1	90.8	90.6	91.2	−1.2

* All values given are mean values ±S. D., shown in parentheses.

of potassium. Preliminary studies on some of these patients fail to demonstrate any increase in urinary excretion of potassium.

As an outgrowth of this previous observation, seven patients in chronic renal failure with hyperkalemia were given single 500 ml. infusions of fat emulsion. As shown in Figure 3, a fairly prolonged lowering of the plasma potassium value to normal level for as long as four hours after the end of the infusion was observed. Since the best that one can ordinarily accomplish with available methods (excluding the use of an artificial kidney) is to obtain a transient and incomplete lowering of potassium in these patients, and since hyperkalemia is not an infrequent cause of death, the therapeutic implications of this observation are obvious.

As stated above, preliminary studies have failed to demonstrate increased potassium excretion to explain this fall. However, since it is generally acknowledged that the hyperkalemia in renal failure is due to the inability of the kidney to excrete potassium, it would seem unlikely that an infusion of fat emulsion would restore this ability to a chronically diseased kidney.

Studies of Lipid Levels and Clearing of Fat

During the course of the electrolyte work, we became interested in the ability of the individual patient to dispose of a given load of infused fat. Originally it seemed logical to suspect that a direct relationship might exist between the degree of cachexia and the rapidity with which fat is removed to badly depleted depots. While we encountered numerous single observations which seemed to confirm this thought, there were also numerous and notable exceptions. Some of these are shown in figure 4. Of particular interest were 6 patients with known diffuse pancreatic disease (3 malignant, 3 inflammatory), all of whom exhibited extremely high and prolonged lipid levels when compared to 25 normal patients, 72 abnormal patients, 9 patients with compensated portal cirrhosis, and 9 patients with diabetes under reasonably good control. Attention is called to the fact that the diabetic patient un-

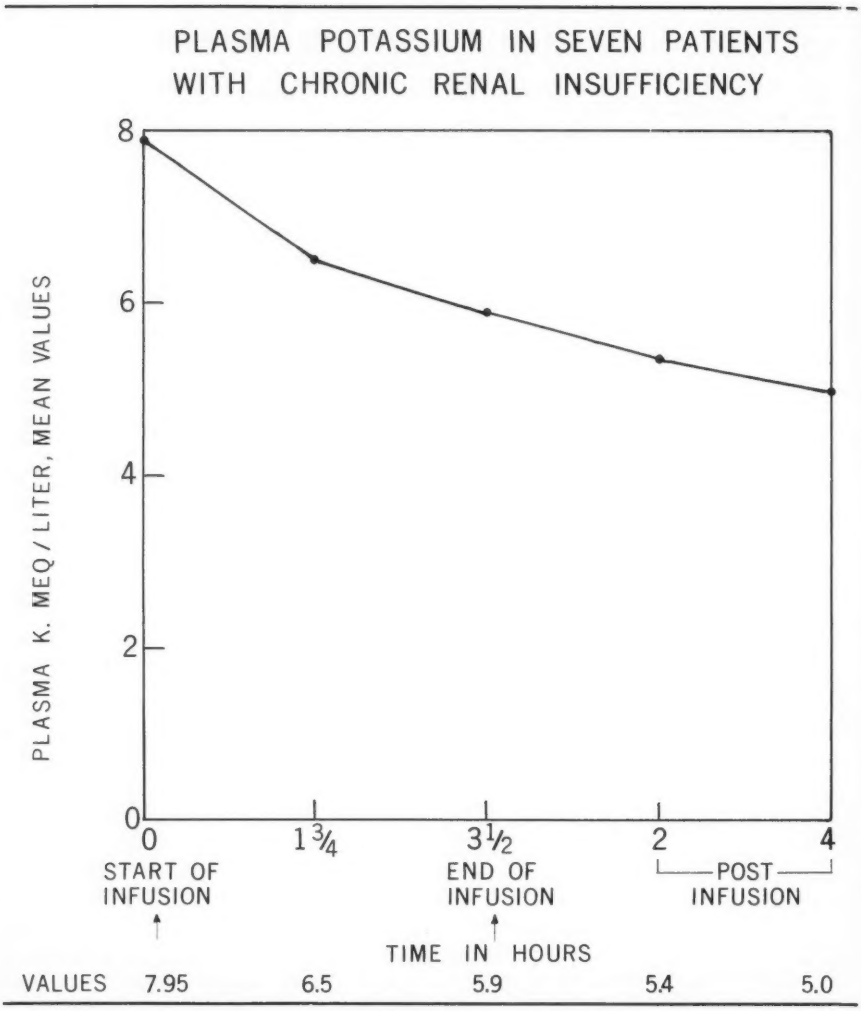


FIGURE 3

der control cleared the infused fat at about the same rate as the normal conrol patients.

It is of further interest that the 9 patients with compensated portal cirrhosis also were slow clearers. Such results seem to conflict with the very significant findings of Baker who reported that patients with cirrhosis have increased amounts of assayable clearing factor.[27] Other groups in this field, particularly Wilkinson and co-workers[28], have reported similar findings with respect to so-called delayed clearers. Such variations in observations merely emphasize the fact that there is a very wide range in the individual ability to remove a fat load from the blood to the peripheral tissues. Whether there exists a common denominator, such as a cachectic nutritional state, to explain delayed clearing is open to speculation. It would seem reasonable that the answer at least in part can be found in some phase of the

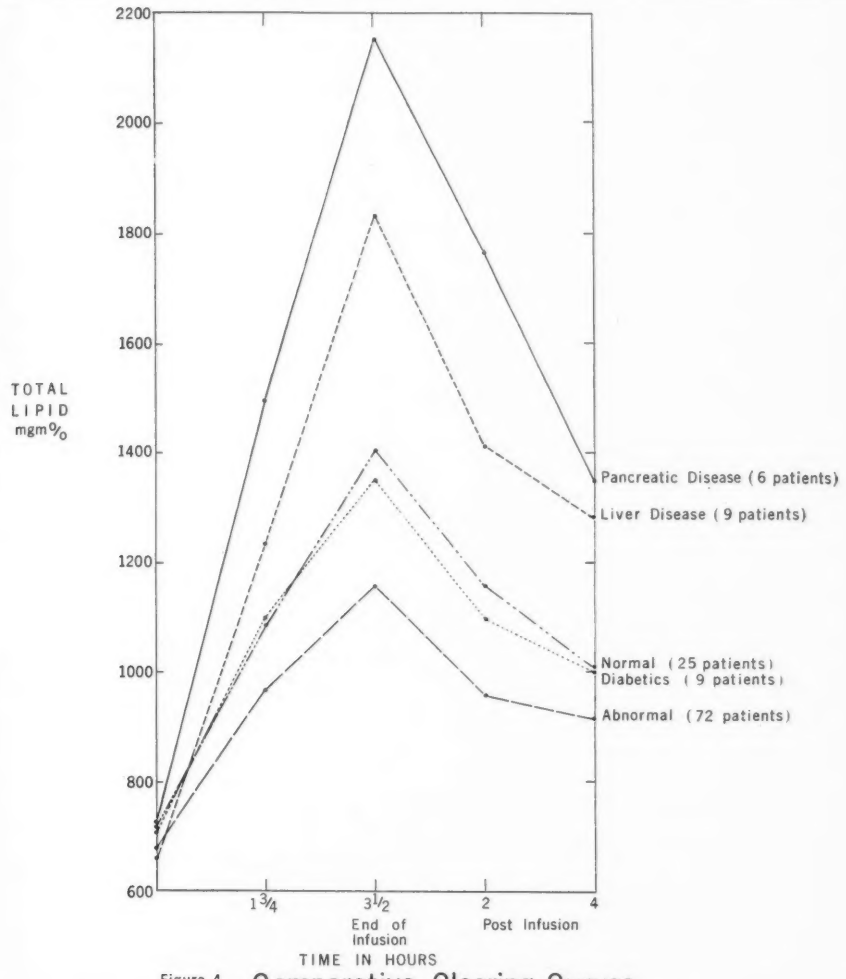


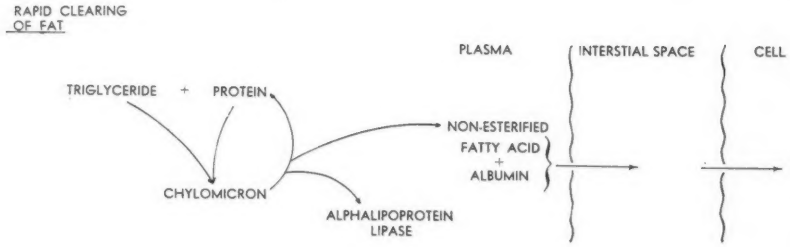
Figure 4 Comparative Clearing Curves

FIGURE 4

clearing mechanism. For this reason a brief review of some of the factors involved might be of some value.

Current Status of the Clearing Factor Concept

In 1943 Hahn observed rather accidentally that intravenous injections of heparin in animals with alimentary lipemia caused a rapid disappearance of the turbidity of the blood.[29] Subsequent work showed that this phenomenon occurs only *in vivo*, but once occurring may continue *in vitro*. The disappearance of the lipemic turbidity is accompanied by a decrease in the total lipid concentration of the blood, indicating that injection of heparin not only transforms turbid lipoprotein into non-turbid forms but also accelerated the removal of lipid from the blood.



After Korn

FIGURE 5

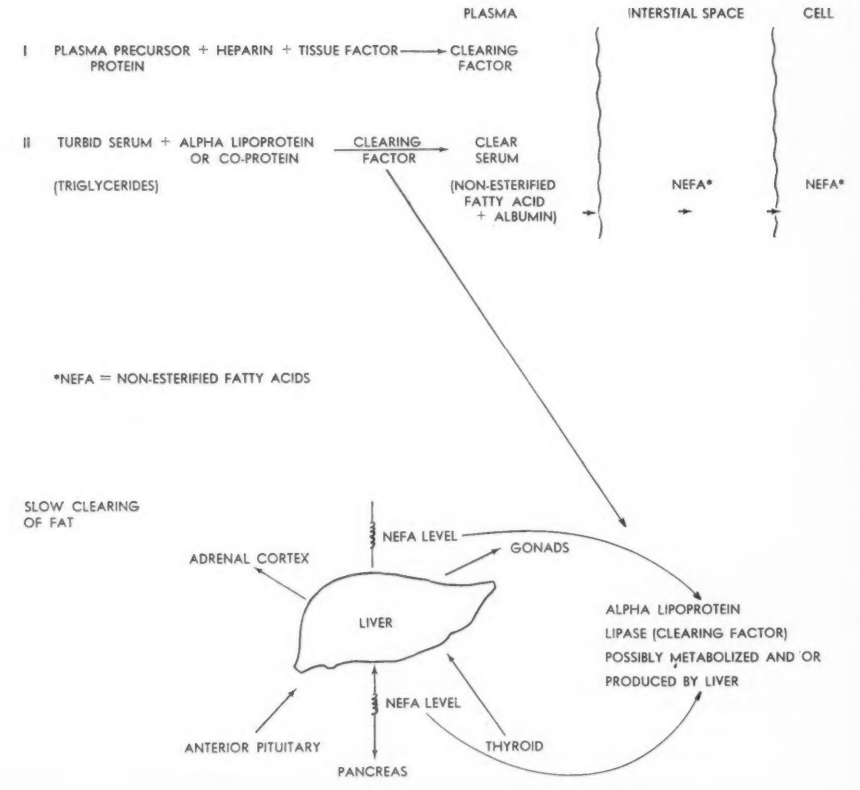


FIGURE 6

While the mechanism of action was not known, clearing factor as such was considered to be the product of an enzymatic system involving both tissue and plasma components in a manner outlined in figure 5.

This scheme was amplified by Korn in 1955,[30] when he demonstrated that normal rat heart muscle contained an enzyme which was identical in its action with heparin-induced clearing factor. This material was subsequently found to be an alpha-lipoprotein lipase which is present in beef and pig heart, lung, spleen, and liver in decreasing quantities and not at all in aorta or muscle. It is distinguished from pancreatic lipase in that it is activated by heparin and deactivated by salt and protamine. The real significance of this work was that Korn showed that clearing enzyme is present and that clearing occurs at the cellular level, but that heparin potentiates this action to the degree that there is an overflow into the blood where the effect is more striking. He further established that the very process of clearing is in a sense a self-perpetuating mechanism, for the very lipoprotein released from the chylomicron during the hydrolytic process of clearing is sufficient to form the substrate for further action by the clearing factor, thus repeating the cycle over again. (Figure 6).

This entire concept assumes considerable importance in the use of intravenous fat when one considers that:

1) The same type of rapid removal of fat under the influence of heparin has occurred when fat is given orally as well as by the intravenous route.

2) That on the basis of available evidence intravenous fat is handled as a foreign body, being removed in part by the reticuloendothelial system. In general, it is found in the lungs, liver, spleen and occasionally the renal tubules as extracellular depositions. However, after heparin this fat is handled more nearly like orally administered lipid.

These observations have strongly suggested that heparin may play an integral and physiological part in fat metabolism. The ultimate fate of the fat removed from the blood under the influence of heparin is of much interest. The removal of injected fat occurs with such rapidity and magnitude that to explain it on the basis of rapid-oxidation seemed unlikely. Furthermore, quantitative studies showed that under the influence of heparin, the concentration of fat in the liver and spleen was actually reduced, and thus these depots could not account for such rapid clearing.

In order to determine the fate of this removed fat, studies were undertaken by our group using a carbon-14 labelled emulsion. The results of these experiments, as shown in tables 4, 5 and 6, demonstrate that under the influence of heparin the following changes occur:

1) A marked rise in respiratory carbon dioxide-C14 and muscle lipid
2) A profound decrease in lipid in the blood and spleen
3) Variable increases in lipid in the skin and intestine.

Fundamentally, heparin **accelerates** the movement of fat from blood to the tissues, and thereby makes fat more rapidly available for oxidative processes. Since one of the basic processes in the whole phenomenon of clearing seems to be lipolysis, and since tissues probably oxidize free fatty acid rather than triglycerides, it seems worth while to speculate that endogenous heparin and/or alpha-lipoprotein lipase play a role in the normal metabolism of fat.

The recent work of Gordon[47] more than ever emphasizes the role of non-esterified fatty acids in the entire scheme of fat transport. The precise mechanisms involved in the production and maintenance of a given level of fatty acid is not completely understood. It seems quite likely that at least part of this control is under strong hormonal influence. Anterior pituitary, pancreatic, thyroid, gonodal, and adrenal relationships have been implicated in both human and animal experiments[48].

Basically the effect of these various hormones on the control of circulating lipids is in the maintenance of the various fractions. Since there is no evidence for oxidative catabolism of lipids in the extracellular compartment, the concentration of lipids in the blood and the rate

TABLE 4

EFFECT OF HEPARIN ON PERCENT C^{14} ACTIVITY* RECOVERED FROM TOTAL ORGANS AND BLOOD ATER INJECTION OF C^{14} SOYBEAN OIL EMULSION.

	Expts. 1 & 2		Expts. 3 & 4		Expts. 5 & 6	
	Heparing	Control	Heparing	Control	Heparing	Control
Liver	23.9	21.8	36.4	17.1	22.5	16.1
Spleen	0.86	4.2	1.70	3.58	0.45	2.06
Lung	1.16	1.53	1.24	0.85	0.49	0.54
Blood	15.8	83.0	8.82	92.3	36.7	91.0

* Per cent Activity Recovered from Organ $= \frac{\text{Cpm x mg. fat in organ}}{\text{cpm x mg. oil injected}} \times 100$

TABLE 5

EFFECT OF HEPARIN ON PERCENTAGE C^{14} ACTIVITY* RECOVERED PER GRAM OF TISSUE AFTER INJECTION OF C^{14} SOYBEAN OIL EMULSION

	Expts. 1 & 2		Expts. 3 & 4		Expts. 5 & 6	
	Heparing	Control	Heparing	Control	Heparing	Control
Muscle	0.112	0.017	0.134	0.034	0.159	0.042
Intestine	0.192	0.133	0.230	0.102	0.311	0.149
Skin	0.137	0.066	0.181	0.139	0.173	0.059

* Percentage C^{14} Activity Recovered Per gm. of Tissue $= \frac{\text{mg. Fat per gm. tisse x cpm}}{\text{mg. Oil Injected x cpm}} \times 100$

TABLE 6

EFFECT OF HEPARIN ON PERCENTAGE C^{14} RECOVERED FROM RESPIRATORY CO_2* AFTER INJECTION OF C^{14} SOYBEAN OIL EMULSION

Pair No.	Control	Heparinized
1	0.70	4.66
2	1.28	2.51
3	1.91	1.84
4	1.16	4.71
5	0.95	3.56

Periods of collection were 30 minutes in each instance.

* Percent $C^{14}O_2$ recovered $= \frac{\mu c\ C^{14}O_2 \text{ collected}}{\mu c\ C^{14} \text{ injected}} \times 100$

at which they are handled may be due to:

1) Changes in the rate of transference between the extra and intracellular lipoprotein.
2) Any factor which influences distribution of lipids between the plasma and the interstial compartment.
3) Changes in blood volume.

It would seem that hormones could easily effect lipid concentration and transport at any one or all of these levels. From a purely speculative standpoint, it might be possible that the very level of non-esterified fatty acids present at a given moment is the regulating device which triggers the sensitivity for the production of varying amounts of clearing factor. In this respect, the level of non-esterified fatty acids would have a role analagous to the thermostatic effect of blood sugar on the release of liver glycogen[49]. Similar to the blood sugar mechanism, one can further postulate that this level of fatty acid is influenced by a fine balance of opposing hormonal forces which ultimately determine the rate at which fat may be transported to peripheral tissues. These hypothetical relationships are illustrated in figure 6.

SUMMARY

There is available for therapeutic use a fat emulsion which can be given intravenously with relative safety. This is not an ideal preparation, but few drugs ever attain complete therapeutic effect without the risk of some unwanted side-effects. When given judiciously and under the proper circumstances, the use of parenteral fat can tide a patient over a nutritional crisis and speed convalescence. Furthermore, this material has made available to the investigator a tool which has been of great value in the study of some aspects of fat metabolism.

REFERENCES

1. Gorens, S. W., Geyer, R. P., Matthews, L. W. and Stare, F. J.: Observations on the use of fat emulsion for intravenous nutrition. J. Lab. and Clin. Med., *34*:1927, 1949.
2. Shafiroff, B. G. P., Mucholland, J. H., Roth, J. H. and Baron, H. C.: Intravenous infusions into human subjects of fat emulsions. Proc. Soc. Exper. Biol. and Med., *70*:343, 1949.
3. Johnson, ., Freeman, S. and Meyer, K. A.: Some effects of intravenous fat emulsions on human subjectWs. J. Lab. and Clin. Med., *39*:176, 1952.
4. Neptune, E. M., Geyer, R. P., Saslow, I. M. and Stare, F. J.: Successful intravenous administration of large quantities of fat emulsion to man. Surg., Gynec. and Obst., *92*: 365, 1951.
5. Mann, G. V., Geyer, R. P., Watkin, D. M. and Stare, F. J.: Fat emulsions for intravenous nutrition in man. J. Lab. and Clin. Med., *34*:699, 1949.
6. McKikkin, J. M., Pope, A., Thayer, S., Ferry, R. M., Jr. and Stare, F. J.: Studies on fat emulsions for intravenous alimentation. J. Lab. U .Clin. Med., *34*:699, 1949.
7. Geyer, R. P., Watkins, D. M., Matthews, L. W. and Stare, F. J.: Parenteral nutrition. XI. Studies with stable and unstable fat emulsions administered intravenously. Proc. Soc. Exper. Biol. and Med., *77*:872, 1951.
8. Becker, G. H. Moeller, H. C. and Grossman, M. I.: Studies on the febrile responses following intravenous fat to human subjects. J. Lab. and Clin. Med., *44*:766, 1954.
9. Moeller, H. C., Grossman, M. I., Palm, L., Cushing, A., Stadler, J. D. and Becker, G. H.: A study of two intravenous fat emulsions in human subjects. J. Lab. and Clin. Med., *46*: 450, 1955.
10. Calloway, D. H. and Spector, H.: Nitrogen balance as related to caloric and protein intake in active young men. Am. J. Clin. Nutr., *2*:405-412, 1954.
11. Benedict, E. P., Woolridge, R. L. and Stepto, R.: The dynamics of protein metabolism. II. The relationship between the level of protein intake and the rate of protein utilization by protein-depleted men and rats. J. Lab. and Clin. Med., *33*:269-279, 1948.
12. Van Itallie, T. B., Moore, F. D., Geyer, R. P. and Stare, F. J.: Will fat emulsions given intravenously promote protein synthesis? Metabolic studies on normal subjects and surgical patients. Surgery, *36*:720-731, 1954.
13. Levey, S., Kreiger, H., Benson, J. W., Davis, J. H. and Abbott, W. E.: Metabolic alterations in surgical patients. IX. The influence of intravenously administered fat emulsions on nitrogen balance in postoperative patients. J. Lab. and Clin. Med., *49*:61-68, 1957.
14. Natelson, S.: Routine use of ultramicro methods in the clinical laboratory. Am. J. Clin. Path., *21*:1153, 1951.
15. Van Slyke, D. D. and Hiller, A.: Determination of chloride in body fluids. J. Biol. Chem., *167*:107, 1947.
16. Kunkel, H. G., Ahrens, E .H., Sr. and Eisenmenger, W. J.: Application of turbidometric methods of estimation of gamma alobulin and total lipid. Gastroenterol., *11*:499, 1948.
17. Peters, John P.: Some remarks on the management of Diabetes mellitus. Yale J. Biol. and Med., *27*:75-89, 1954.

18. Geyer, R. P.: Verbal report. Meeting of the Surgeon General's Task Group for the Study of Intravenous Fat Alimentation, October, 1956, Denver.
19. Hadley, June 5. and Meng, H. C.: Pancreas and the production of lipemia clearing factor. Proc. Am. Soc. Stud. Arteriosclerosis, *3*:479-506, 1956.
20. Mueller, John: Verbal report. Meeting of the Surgeon General's Task Group for the Study of Intravenous Fat Alimentation, October, 1956, Denver.
21. Lambert, G. F., Miller, J. P. and Frost, D. V.: Febrile response following intravenous administration of fat. Am. J. Physiol., *164*:490, 1951.
22. Becker, G. H. and Grossman, M. I.: Studies on the thermogenic response to intravenous fat emulsions. J. Lab. and Clin. Med., *43*: 752, 1954.
23. Moeller, H. C., Haynes, P. C., Bernstein, L., Levy, L. and Grossman, M. I.: Intravenous administration of fat emulsion in human subjects. Med. Nutrition Lab. Report No. 163, April 15, 1955.
24. Loewy, A., Freeman, L. W., Marcello, A. and Johnson, V.: Increased erythrocyte destruction on a high fat diet. Fed. Proc., *1*:25, 1942.
25. Freeman, L. W., Loewy, A., Marchello, A. and Johnson, V.: Increased total bile pigment output on a high fat diet. Am. J. Physiol., *138*:230, 1943.
26. Creditor, M. C.: Some observations on effects of intravenous fat emulsions on erythrocyte fragility. Proc. Soc. Exper. Biol. and Med., *82*:83, 1953.
27. Baker, S. P., Levine, H., Turner, L. and Dubin, A.: Lipoprotein lipase response in Caennec's cirrhosis. Proc. Soc. Exp. Biol. and Med., *99*:670, 1958.
28. Bozian, R. C., Davidson, N. W., Stutman, L. J. and Wilkinson, C. F.: Observations on the use of intravenous fat emulsions in man. Metabolism, Vol. VI, No. 6,703, 1957.
29. Hahn, P. F.: Abolishment of alimentary lipemia following injection of hesparin. Science, *98*:19-20, 1943.
30. Korn, E.: Clearing factor, a heparin activated lipoprotein lipase. J. Biol. Chem., *215*:1-15, 1955.
31. Anderson, N. C. and Fawcett, B.: An antichylomicronemic substance produced by heparin injection. Proc. Soc. Exper. Biol. and Med., *74*:768-771, 1950.
32. Anfinsen, C. B., Boyle, E. and Brown, R. K.: The role of heparin in lipoprotein metabolism. Science, *115*:585-586, 1952.
33. Swank, Rav L. and Levy, S. W.: Chylomicron dissolution. Dosage and site of action of heparin. Am. J. Physiol., *171*:208-217, 1952.
34. Boyle, E., Bragdon, J. H. and Brown, R. K.: Role of heparin in *in vitro* production of alpha lipoproteins in human plasma. Proc. Soc. Exper. Biol. and Med., *81*:475-477, 1952.
35. Grossman, M. I. and Strub, I. H.: Effect of heparin on the fate of intravenously administered fat emulsion in rats. Proc. Soc. Exper. Biol. and Med., *85*:356-359, 1954.
36. Hertzstein, J., Wang, C. I. and Aldersberg, D.: Effect of heparin on plasma lipid partition in man: Studies in normal persons and in patients with coronary atherosclerosis, nephrosis and primary hyperlipemia. Ann. Int. Med., *40*:290, 1954.
37. Brown, W. D.: Reversible effects of anticoagulants and protamine on alimentary lipoemia. Quart. J. Exper. Physilo., *37*:75, 1952.
38. Lerner, S. R., Chaikoff, I. L., Entenman, C. and Dauben, .: The fate of C^{14} labelled palmitic acid administered intravenously as a tripalmitin emulsion. Proc. Soc. Exper. Biol. and Med., *70*:384, 1949.
39. Geyer, R., Chipman, J. and Stare, F. J.: Oxidation *in vivo* of emulsified radioactive trilaurin administered intravenously. J. Biol. Chem., *176*:1469, 1948.
40. Rall, T. W., Becker, G. H. and Scully, N. J.: The biosynthesis of uniformly labelled C^{14} soybean oil. Report No. 122, Med. Nutrition Lab., 1953.
41. Soderhjeim, Ulla and Soderhjeim, Lars: Fat distribution in feces using Mojonnier extraction flasks. J. Lab. and Clin. Med., *34*:1941, 1949.
42. Lewis, A. E., Goodman, R. D. and Schuck, E. A.: Organ blood volume measurements in normal rats. J. Lab. Clin. Med., *39*:704, 1952.
43. Bragdon, J. H. and Havel, R. J.: *In vivo* effect of anti-heparin agents on serum lipids and lipoproteins. Am. J. Physiol., *177*:128, 1954.
44. Pierce, F. T.: The interconversion of serum lipoproteins *in vivo* metabolism. *3*:142, 1954.
45. Levy, S. W. and Swank, R. L.: The effect of *in vivo* heparin on plasma esterase activity and lipoemia clearing. J. Physiol., *123*:301, 1954.
46. Hewitt, J. E., Hayes, T. L., Gofman, J. W., Jones, H. B. and Pierce, F. T.: Effects of total body irradiation upon lipoprotein metabolism. Am. J. Phvsiol., *172*:579, 1953.
47. Gordon, R. S.: Unesterified fatty acid in human blood plasma. J. Clin. Invest., *35*:210, 1957.
48. Boyd, G. S. and Oliver, M. F.: Hormonal control of the circulating lipids. British Med. Bull., *14*:239, 1958.
49. Soskin, S. S. and Levine, R.: Carbohydrate metabolism. U. of Chicago Press, 1946.

THE PARAPROTEINS *

L. MICHAEL GLICK, M.D. and DONALD M. GELB, B.A.*****

The term dysproteinemia[1] is often used to refer to those disease states in which elevated values of normally occurring plasma globulins are noted. In a few diseases, however, the hyperglobulinemia results from the presence of an abnormal protein and in these instances it is referred to as paraproteinemia[1].

Interest in the "paraproteins" can be traced back to 1845, when MacIntyre and Watson[2] described a grocer who had very fragile bones and a peculiar urine charged with "animal matter." A sample of this patient's urine, which Watson sent to Sir Henry Bence-Jones for analysis, was accompanied with the following note: "The tube contains urine of a very high specific gravity . . ., when boiled it becomes highly opaque . . ., but as it cools, it assumes the consistence and appearance which you now see; heat reliquifies it. What is it?"

Almost a century elapsed after these initial observations before any further work on paraproteins was undertaken. In part, this delay can be attributed to the fact that methods of protein analysis and study had yet to be developed.

Howe, in 1921[3], utilizing the observation that plasma proteins are precipitated from aqueous solutions by high concentrations of neutral salts, was the first worker to separate and classify proteins by virtue of this "salting out phenomenon." By varying the concentration of the salt used, Howe was able to fractionate plasma into euglobulins, pseudogloblulins and albumins. Although he used sodium sulfate for his separations, it was subsequently shown that other neutral salts, such as ammonium sulfate, magnesium salts and phosphatases could also be used.

Investigation of plasma proteins was further advanced in 1937 by the introduction of the electrophoretic method devised by Tiselius[4], who based his method on the principle that different fractions of proteins migrate with different velocities in an electric field[5]. A protein solution (at a specified pH), is placed at the bottom of a U tube (Figure 1). A buffered solution, into which two electrodes are introduced, is then layered over the protein solution. The rate of migration of the protein fractions in the electric field produced may now be observed.

As most protein solutions are colorless, the moving protein fractions are visualized by optical systems utilizing the principle that the interphase between two fractions focuses light by virtue of its refractive index. Thus, the different protein fractions appear as peaks of focused light, and the amount of each protein fraction can be determined by photographing and then measuring the area under each peak.

A normal descending pattern for adult serum is shown in Figure 2. Albumin, (A), accounts for about 65% of the total serum protein. The alpha globulins, alpha 1 and alpha 2, represent about 4% and 8%, respectively, of the total protein. The beta globulin comprises about 10%, and the gamma globulin makes up the remaining 13% of serum protein. If plasma instead of serum is analyzed, the fibrinogen forms a peak between the beta and gamma globulin fractions and represents about 4% of the plasma proteins.

The fraction designated the "euglobulin" by the Howe method corresponds to the beta and gamma globulin of the electrophoretic pattern. The pseudoglobulin by the Howe method, corresponds to the electrophoretic fractions of albumin and the alpha 1 globulin.

Recently, electrophoresis has been simplified by techniques utilizing filter paper or starch blocks as the vehicles for protein migration[6]. By these methods,

* From the Departments of Medicine of The Chicago Medical School and the Mount Sinai Hospital.

** Instructor, Department of Medicine, The Chicago Medical School; Chief Resident, Department of Medicine, Mount Sinai Hospital.

*** Senior Medical Student, The Chicago Medical School.

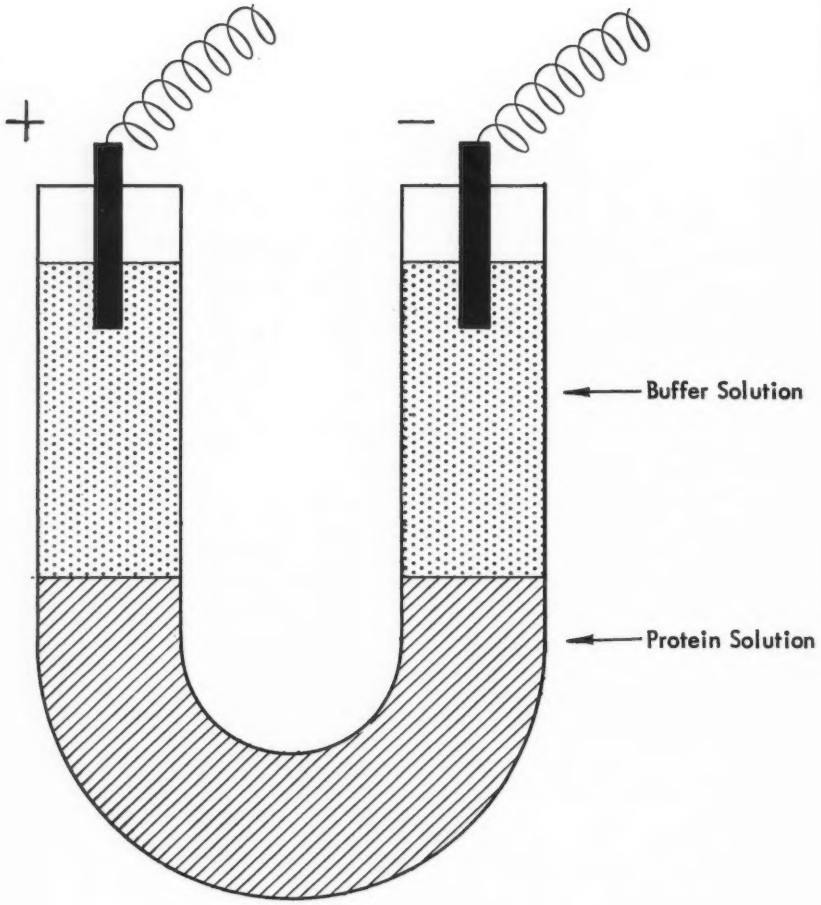


FIGURE 1

optical systems are eliminated as the proteins can be strained and measured directly.

With Svedberg's intorduction of the ultracentrifuge in 1940[7], a great advance in the study of the plasma proteins was achieved. The rate at which a particle in solution settles under the action of centrifugal force depends on 1) the density and viscosity of the solution, 2) the size, shape and density of the particle (a specific property of the molecule), and 3) the force applied. Due to the minute size of protein molecules, great amounts of force (400,000 times gravity) must be applied over a long period of time to affect adequate separation.

These complex time-force-distance relationships are expressed as Svedberg units (S). The range of sedimentation units routinely measured by analytical ultra centrifugation procedures extends from about 2 S for small proteins to about 1,000 S for some bacteriophages. The value for albumin is 4.5 S, globulin 7 S while proteins with values exceeding 15 S are referred to as macroglobulins[1].

As these methods for protein study became more widely known, attention was focused on the paraproteins in disease.

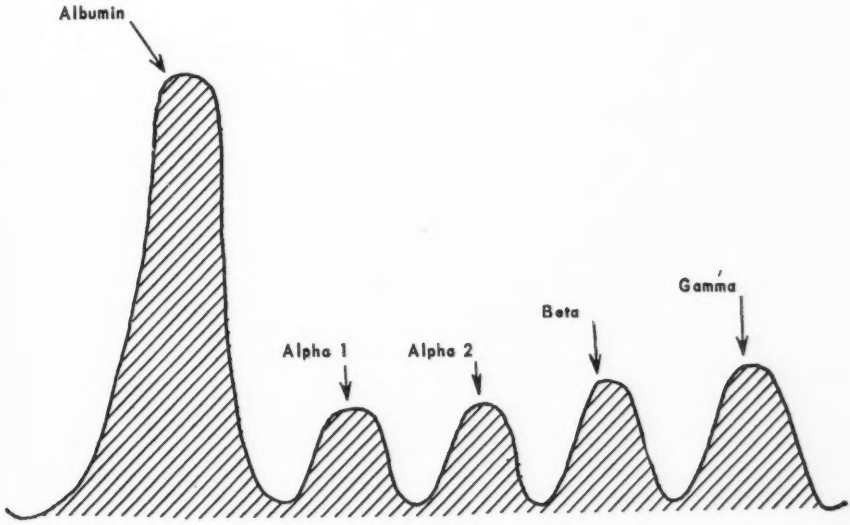


FIGURE 2

Wintrobe and Buell[8], in 1933, while studying a patient with multiple myeloma, described a globulin which precipitated when cooled and redissolved upon warming. Fourteen years later, Lerner and Watson[9] introduced the term cryoglobulin to describe proteins with this abnormal physical characteristic.

Subsequently, it was demonstrated that small quantities of cryoglobulin could be found in the serum of patients with disease states other than multiple myeloma. These other diseases include: kala azar, malaria, lupus erythematosus, rheumatoid arthritis, periarteritis nodosa, lymphosarcoma, lymphatic leukemia, polycythemia vera, cirrhosis and subacute bacterial endocarditis[10]. Cryoglobulinemia sufficiently severe to produce signs and symptoms is most often associated with multiple myeloma. Recently however, patients with essential cryoglobulinemia have been reported[10, 11, 12].

Whether the cryoglobulinemia is secondary or primary, the common denominator appears to be a disturbance of the reticulo-endothelial system. This may be neoplastic disease, with increased production of abnormal globulins; infectious, with abnormal immunological response; or some other, as yet undefined, mechanism[10, 13]. Efforts to correlate cryoglobulin production with abnormal cellular morphology in the reticulo-endothelial system have not yielded constant results[1, 14]. Abnormal appearing plasma cells are frequently encountered in some patients with cryoglobulinemia; in other cases, however, despite massive cryoglobulinemia, the plasma cells appear quite normal.

Putnam[13] has demonstrated that although these cryoglobulins are similar to normal gamma globulin, their end-group amino acids differ. Normal gamma globulin has both N-terminal aspartic and glutamic acid radicals, while the cryoglobulins have (a) only terminal aspartic groups, (b) only terminal glutamic groups, or (c) both glutamic and aspartic as terminal amino acids, but twice the usual amount.

Furthermore, Putnam[6, 13] has demonstrated that there is sometimes attached to the cryoglobulin an undefined carbo hydrate fraction (PAS positive). It has been postulated that this cryoglobulin-carbohydrate complex may be involved in the formation of paraamyloid, a material occasionally deposited in the tis-

sues of patients with multiple myeloma. This form of amyloidosis closely resembles the "primary" type in that it is deposited in the heart, respiratory tract, gastro-intestinal tract and blood vessels.

Beyond these biochemical differences and the unexplained cold precipitation, there is no specific difference between the cryoglobulins and normal proteins. Electrophoretic studies[6, 10, 12] have shown that cryoglobulin migrates most commonly with the gamma fraction, although "alpha and beta migrations" have also been described[1]. Ultracentrifugation indicates that cryoglobulin has the molecular weight of gamma globulin with the major component near 7 S.

Marked cryoglobulinemia is often but not invariably characterized by a definite clinical syndrome[1, 10, 11, 12]. When present, the primary clinical features are intolerance to cold, as manifested by Raynaud's phenomena, purpura, urticaria, ulceration of the skin, peripheral gangrene and retinal vascular stasis. It has been postulated that these symptoms are largely due to precipitation of cryoglobulin in peripheral blood vessels[10, 12]. Volpe[10] proposed a second mechanism to explain some of the clinical findings in one patient he observed. He felt that the cryoglobulin, acting as a foreign protein introduced into the body, set up a local tissue reaction. This latter theory has received some support in the work of Heller et al[15] who has observed the phagocytosis of cryoglobulins by leukocytes, even when these proteins are present in minute quantities.

It appears likely that the following three factors determine the production of symptoms in these cases: 1) the amount of cryoglobulin present; 2) its solubility characteristics in relation to temperature; and 3), the interaction of cryoglobulin with other plasma proteins[10].

Whether or not precipitation of cryoglobulin commonly occurs in deep vessels is uncertain. Pulmonary arteriolar obstruction with pulmonary hypertension has been attributed to cryoglobulins[16]. Several patients with cryoglobulinemia have died in uremia[10], raising the possibility of renal vascular precipitation of cryoglobulin. In the case described by Cugudda[17] multiple visceral thrombosis associated with cryoglobulinemia was reported.

Another paraprotein was uncovered by Waldenstrom in 1944[18], who applied the term macroglobulin to a large protein with a molecular weight in excess of 1,000,000 and a Svedberg determination of 19.2. As this protein was found in a patient with cryoglobulinemia a great deal of confusion ensued.

Subsequent electrophoretic studies[1, 12] have demonstrated that pathological macroglobulins migrate as a peak between the beta and gamma fractions and are labelled the "M" fraction. It has also been shown that some apparently normal individuals have small quantities of macroglobulin (less than 5%) migrating in the alpha range. It has been suggested that macroglobulins may be polymers of normal gamma globulins[19]. The polymer linkage involved appears to be in the nature of a sulfur-sulfur bond, since methionine and cystine terminals have been demonstrated.

Patients with Waldenstrom's syndrome of macroglobulinemia[1, 12, 13, 19], characteristically suffer from lassitude, dyspnea, epistaxis and mucosal bleeding. Examination frequently reveals pallor, edema, slight hepatosplenomegaly and painless mild lymph adenopathy.

The pathologic physiology of cryoglobulinemia and macroglobulinemia may be illustrated as follows:

Infection is common in both conditions and Mackay[12] believes infection is related to faulty antibody formation resulting from competitive use of amino acids for synthesis of abnormal protein. The bleeding phenomena[11, 12, 13] sometimes evident in patients with cryoglobulinemia has been ascribed to the cryoglobulin interfering with fibrin formation. Another factor might be the damaging effect that precipitating cryoglobulin has upon the capillary walls. The macroglobulins appear to disrupt the normal clotting mechanism by absorbing factor V. A thrombocytopenia may occur in either condition.

Finally, it should be noted that Sjogren's syndrome[20], (dryness of mucous membranes in eyes, nose, mouth and vagina) has been reported in several cases associated with cryoglobulinemia

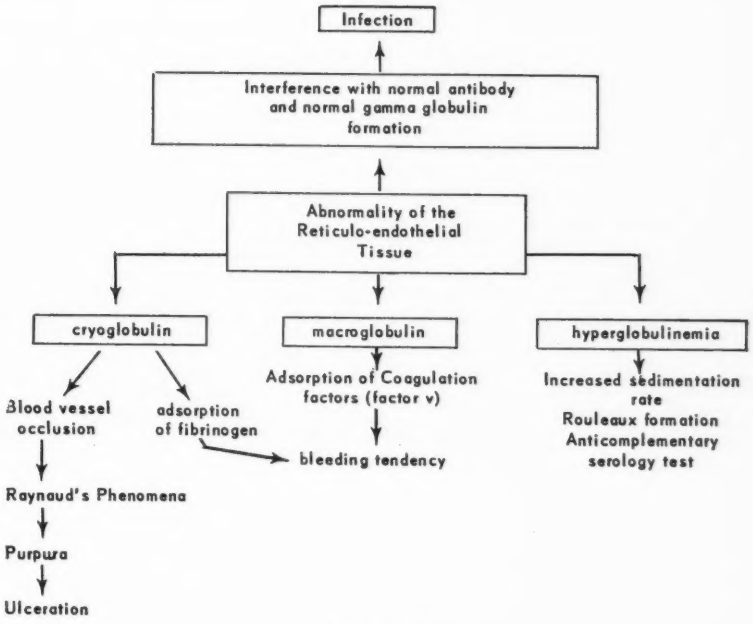


FIGURE 3

or macroglobulinemia.

Pyroglobulin[21, 22], another paraprotein, has been isolated in patients with multiple myeloma. The name was applied by Martin[22] to an abnormal golbulin which precipitated from sera heated to 56° C. Like macroglobulin, it migrates between the beta and gamma globulin fractions. Further studies will be needed to understand this paraprotein and to clarify its possible relationship to the Bence-Jones protein in the urine.

It is interesting that although the Bence-Jones protein was the first paraprotein uncovered, it is perhaps the least clearly understood.

It is found in the urine in about 50% of the cases of multiple myeloma, having the peculiar property of forming a white, cloudy precipitate at temperatures of 50° to 60° c., going back into solution at about the temperature of boiling and then reappearing on cooling[6, 13]. This protein is very erratic in its thermal behavior since it does not always redissolve upon heating. Putnam[6] considers it to be endogenous in origin and to be very rapidly excreted in the urine, so that it is rarely demonstrable in the serum. The spherical shape of the protein and its small size may be responsible for this rapid elimination. Attempts to further elucidate the nature of Bence-Jones protein by amino acid analysis have been unsatisfactory.

Recently, cryofibrinogen, pyrofibrinogen and cryofibrinolysis[1, 12] have been described. Their position in the spectrum of disorders of protein metabolism is still not clear.

BIBLIOGRAPHY

1. Waldnsrom, J.: Abnormal Proteins in Myeloma. Adv. Int. Med., 5:398, 1952.
2. Bence-Jones, H.: Lancet, 2:88, 1847.
3. Howe, P. E.: Use of Na_2So_4 as the Globulin Precipitant in the Determination of Proteins in Blood. J. Biol. Chem., 49:93, 1921.
4. Tiselius, A.: New Apparatus for Electrophoretic Analysis of Colloidal Mixtures. Trans. Faraday Soc., 33:524, 1937.
5. West and Todd: Biochemistry, Macmillan Company, New York, 1951.

6. Putnam, F. W.: Physiochemical Study of Serum Proteins. J. Biol. Chem., *202*:727, 1953.
7. Svedberg, T. and Pedersen, K. O.: The Ultracentrifuge, Oxford University Press, London, 1940.
8. Wintrobe, M. M. and Buell, M. V.: Hyperproteinemia Associated with Multiple Myeloma. Bull. Johns Hopkins Hosp., *52*:156, 1933.
9. Lerner, A. B. and Watson, C. J.: Cold Precipitable Serum Globulin. Am. J. Med. Sci., *214*:416, 1947.
10. Volpe, R., Bruce-Robertson, H., Fletcher, A. H. and Charles, W. B.: Essential Cryogloubulinemia. Am. J. Med., *20*:533, 1956.
11. Firkin, B. G.: Essential Cryoglobulinemia. Am. J. Med., *24*:974, 1958.
12. Mackay, I. R., Eriksen, N., Motulsky, A. G. and Voliwider, W.: Cryo and Macroglobulinemia. Am. J. Med., *20*:564, 1956.
13. Putnam, F. W.: Proteins in Multiple Myeloma. Arch. Biochem. & Biophysics, *65*:39, 1956.
14. Barr, D. P., Reader, G. G. and Wheller, C. H.: Cryoglobulinemia. Am. Int. Med., *32*:6, 1950.
15. Heller, P. et al.: Phagocytosis of Cryoglobulin. Am. J. Med. Sci., *236*:208, 1958.
16. Muirhead, E. E., Montgomery, P. O. and Gordon, C. K.: Thromboembolic Pulmonary Vascular Sclerosis. Arch. Int. Med., *89*:41, 1952.
17. Cugudda, P.: Plasmocitoma con Crioglobulinemia c Trombosi Arteriose e Venose Multiple. Minerva Med., *2*:205, 1952.
18. Waldenstrom, J.: Incipient Myelomatosis—A New Syndrome? Acta Med. Scandinav., *117*: 216, 1944.
19. Glenchur, H., Zinneman, H. H. and Briggs, D. R.: Macroglobulinemia. Ann. Int. Med., *48*:1055, 1958.
20. Morgan, L. M.: The Probable Systemic Nature of Mikulicz's Disease and Its Relation to Sjogren's Syndrome. N.E.J.M., *251*:5, 1954.
21. Branchfield, J. and Myerson, R. M.: Pyroglobulinemia: A Diagnostic Clue in Multiple Myeloma. J.A.M.A., *161*:865, 1956.
22. Martin, W. J. and Mathieson, D. R.: Pyroglobulinemia. Proc. Staff Meet. Mayo Clinic, *28*:545, 1953.

BASIC QUESTIONS RELATING TO THE CHRONIC USE OF THE ANTI - INFLAMMATORY STEROIDS

RACHMIEL LEVINE, M.D.*

The adrenal cortex is capable of producing and secreting a variety of steroids. The normal end products of this synthetic machinery in the human are, in the main, the following hormones:

1. **Aldosterone,** the chief physiological function of which seems to be a preservation of normal excretion patterns of sodium and potassium;

2. **Hydrocortisone** and **Corticosterone,** which have a perceptible but relatively minor role in water and electrolyte balance; the main effect lies in their control of certain metabolic reactions and in their ability to protect the body against a host of non-specific stressor agents;

3. **Androgenic steroids,** the actions of which are qualitively indistinguishable from the androgens of the testes.

The so-called anti-inflammatory or antiphlogistic action of certain steroids was discovered by Hench, Kendall and their associates in 1949. It soon became apparent that this therapeutic effect was restricted to Group 2 of the adrenal steroids, the group which has often been called either the metabolic steroids or the gluco-corticoids. A potent mineralocorticoid like desoxycorticosterone has no anti-inflammatory action whatever; neither do any of the androgens.

We do not, even at present, understand completely the exact mechanisms of action involved in the anti-inflammatory effect, but it is possible to understand quite well why this effect is so intimately related to the metabolic activities of the steroids. Histological and histochemical and purely chemical studies seem to agree that active steroids inhibit the proliferation of cells (especially fibroblasts) in an inflammatory zone. The intense activity in forming new cells and intercellular material is diminished or practically abolished. The action is exerted locally, as has been amply demonstrated by the local instillation of steroids.

While this action is useful in preventing the signs and symptoms of inflammation, it is understandable why spread of infection would be encouraged from a focus which is not effectively walled off. This anti-proliferative, anti-anabolic action may be seen even in the absence of an allergic or inflammatory focus. Thus it was known for some years that in Cushing's disease, the chronic exposure of the body to large amounts of gluco-corticoids leads to a loss of cells and intercellular material, which is especially pronounced in the subcutaneous tissues and in bone. The result is the thin skin and the osteoporosis characteristic of the syndrome. It was also known that patients with this syndrome were prone to septicemias due to spread from locally infected areas.

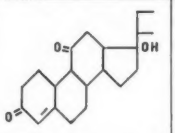
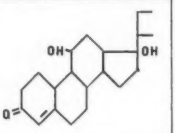
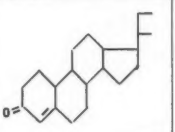
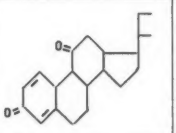
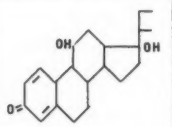
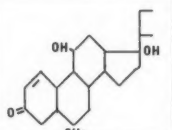
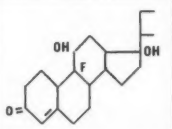
If we examine the nature of the metabolic action of the gluco-corticoids, it becomes evident that their effects on carbohydrate metabolism are not direct, and that the main metabolic activity seems to be exerted in the area of making more protein available for conversion to carbohydrate: again a catabolic or anti-anabolic action. We see, therefore, a clear hint in the picture of these relationships; namely, that in all likelihood the mechanism of metabolic activity and the mechanism of anti-inflammatory activity are closely related, if not identical.

The chronic administration of potent steroids for weeks, months, and years leads to so-called "side effects." all of them undesirable. They are: edema with sodium retention, weakness with potassium loss, osteoporosis and fractures, bleeding or perforation of peptic ulcers, spread of infection to viruses, bacteria and fungi, etc. It would

* Professor of Medicine, The Chicago Medical School; Chairman, Department of Medicine, Michael Reese Hospital.

of course, be of inestimable value for the physician to have at his disposal a steroid which he could use chronically and which would possess desirable anti-inflammatory action and be free or almost so, from the undesirable "side effects." This is clearly impossible for most of the effects listed. For example, if a material is to have an inhibitory action on the formation of inflammatory tissue, it will have to result in the promotion of microbial spread from a focus which is not walled off. This is not a side effect, it is "the" anti-inflammatory effect.

Again, whatever be the etiology of a peptic ulcer, the circulation in the body of anti-inflammatory substances will

		STRUCTURE	ANTI-INFLAMMATORY ACTIVITY	METABOLIC ACTIVITY	MINERALOCORTICOID ACTIVITY
1	CORTISONE		100*	100*	100*
2	HYDROCORTISONE		125	125	100
3	DESOXYCORTICOSTERONE		1	1	2500
4	METICORTIN		400	400	0-2
5	METICORTOLONE		500	500	0-2
6	6—∝ METHYL PREDNISOLONE		600	500	0
7	9—∝ FLURO-HYDROCORTISONE		1200	1200	30,000

*CORTISONE IS THE STANDARD OF COMPARISON. ITS POTENCY IS ARBITRARILY PUT AT 100.

clearly sooner or later lead to a weakening of the new fibrous tissue with which the body attempts to heal the defect. Parenterally administered steroids cannot, of course, be told to exert their anti-inflammatory effect in one area and to stay away from a latent peptic ulcer or a half walled-off tuberculous focus. It seems also fairly evident that a similar action is involved in the suppression and weakening of osteoid tissue with the subsequent rarefaction of bone and proneness to fracture.

Retention of water and minerals seems to have its basis in a different kind of primary action from the effects discussed above, and we have seen that there exist steroids like desoxycorticosterone which only possess this action and little or no metabolic activity. The hope of being able to change the steroid molecule in such a way as to do away with the electrolyte retention was foreseen and actually accomplished.

If one removes hydrogens from the bond between carbon 1 and carbon 2 of the nucleus (Fig. 1) of the cortisone and hydrocortisone molecule, one gets the group of so-called meti or 1- dehydro steroids. By bioassay and from clinical experience these compounds have four to five times the anti-inflammatory activity of the parent material per milligram, and extremely little or practically no action on the sodium and potassium balance. This is also true, if, in addition, a methyl group is added in the 6- position. On the other hand, if a halogen atom such as fluorine is substituted in position 9-, then the electrolyte retaining effect becomes enormously enhanced.

A great amount of work has been done in the production and testing of all types of variants of the steroid molecule, and a survey of the results makes it apparent that it is possible to produce more potent anti-inflammatory steroids than the original cortisone and that it is also possible to enhance, reduce or abolish the water and electrolyte effects of such steroids. But it would seem that hand in hand with the anti-inflammatory activity go the glucocorticoid and general metabolic actions and effects. This bears out the view that the metabolic and anti-inflammatory effects are most probably the results of the same basic action.

How does the above discussion relate to the choice of the proper steroid in those instances in which an anti-inflammatory and anti-allergic effect is desirable? The most suitable steroids for current use seem to be the meti-compounds and their 6- methyl derivatives, since those side effects which depend on electrolyte and water retention may be abolished or greatly diminished. There is no advantage in the use of certain steroids simply because they are more potent. That is, activity per milligram, is not synonymous with improvement. On the contrary, it becomes more difficult in practice to manipulate and establish minimum maintenance dosage the more potent the steroid is.

From the standpoint of investigation it is, of course, interesting and enlightening to test a great variety of steroids. Only in this way can one gain an appreciation of the relation between chemical structure and biological activity. The difficulties arise when it is claimed on the basis of short time chemical trial that this or that steroid is "superior," has less "side effects," etc. The general public as well as the medical profession is under pressure to use the "newest" and most "potent" of the agents, before the primary evidence is sufficiently checked.

Steroids are large-caliber weapons and should not be aimed at small targets. It is extremely satisfying to be able to relieve a patient with rheumatoid arthritis of his crippling symptoms, but this satisfaction sours and curdles when vertebrae collapse, ulcers bleed, muscles weaken or mycotic infection takes hold. It is therefore in practice the better part of wisdom to use steroids sparingly and when forced to do so, to use these compounds which are reasonably free of electrolyte effects.

COMPREHENSIVE RECENT REFERENCES

1. Soffer, L. J. and Orr, R. H.: Biological and Clinical Investigations of Newer Hydrocortisone Analogues. Metabolism, 7:383-573, 1958.
2. Fried, J. and Borman, A.: Synthetic Derivatives of Cortical Hormones, Vitamins and Hormones. Volume 16, pp 303-369, 1958.

PROLONGED STEROID THERAPY AND ITS IMPLICATIONS

JAY J. GOLD, M.D.*

The realization that certain adrenal steroids were capable of inhibiting the pituitary elaboration of adrenocorticotropin, thereby causing adrenal atrophy, dates back to animal studies done at least twenty years ago[1]. Furthermore, it was clinically appreciated that in Cushing's syndrome due to adrenal adenoma, the contralateral adrenal often was atrophic[2]. This was presumably due to the same mechanism demonstrated in animals. However, it was not until recent years when cortisone and subsequently newer and more potent steroids became available for general clinical use (discriminate and indiscriminate) that the sequella of adrenal suppression atrophy became apparent[3].

Pituitary-Adrenal Physiology

Normally, in the maintenance of pituitary-adrenal homeostasis, the pituitary elaborates adrenocorticotropin (ACTH) which then stimulates the adrenal gland to produce cortisol (hydrocortisone), a major physiologic product of adrenal cortical function. When cortisol is produced in amounts greater than required by the tissues, the excess is capable of inhibiting further elaboration of ACTH as shown by the dotted line in Fig. I. This serves as a cause and effect check mechanism, thereby maintaining the normal homeostasis.

The cortisol that is produced is secreted into the blood stream where presumably it may then act directly at the tissue level, allowing the tissues to function normally according to the permissive action of adrenal steroids expounded by Ingle[4] and others[5,6]. Much of it is carried to the liver where it is transformed into its excretory products, tetrahydrocortisol, tetrahydrocortisone and their glucuronide esters. These are returned to the blood stream and eventually excreted in the urine as these products, though small amounts of cortisol and cortisone may also be found in the urine. This is what is measured as urinary 17-hydroxycorticosteroids and serves as an index of adreno-cortical function. Newer methods allow the measurement of the 17-hydroxycorticosteroids directly in the blood, thereby giving a more direct index of adrenocortical function.

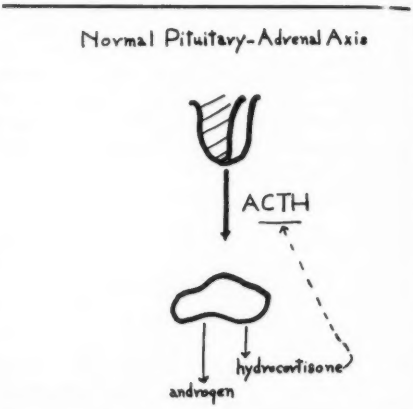


Fig. I. Normal pituitary-adrenal axis. Reciprocal effects of ACTH and hydrocortisone.

Fig. II shows the most abundant urinary corticoids that are found. Cortisol and cortisone are found in smallest quantity. Tetrahydrocortisol and tetrahydrocortisone, along with alpha and beta cortol and cortolone, comprise the major fraction of the total urinary corticoids that are measureable. Cortisone and cortisol are the active forms of the steroids, whereas the reduction products are virtually inactive and are excreted as such.

The normal homeostatic mechanisms are such that the pituitary-adrenal thermostat is set at a level whereby ACTH and cortisol are produced in amounts just adequate to satisfy tissue needs. In situations of stress this thermostat is set at a higher level. In certain disease

* Director, Department for Research in Human Reproduction and Attending Physician, Michael Reese Hospital: Clinical Assistant Professor of Medicine, The Chicago Medical School.

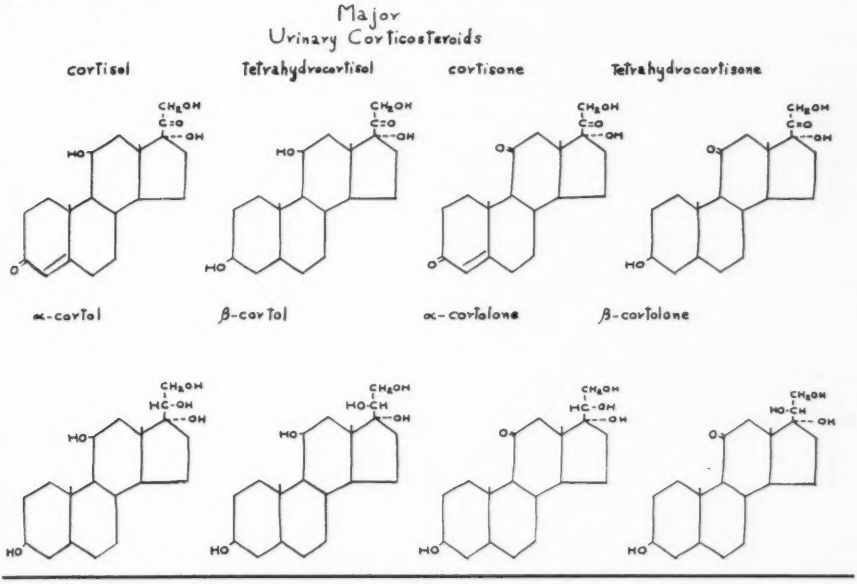


Fig. II. Major urinary 17-hydroxycorticosteroids.

states, as in Cushing's syndrome, the thermostat may also be set at a higher functioning level. As may be seen in Fig. III, when exogenous steroid in the form of cortisone or cortisol is administered to an individual, an amount of steroid is presented to the blood stream and to the pituitary over and above what would normally be produced. This elevated quantity then is capable of inhibiting further pituitary elaboration of ACTH. The net effect of inhibiting pituitary stimulation of adrenal function (by inhibiting ACTH) is that the adrenal becomes atrophic. This is represented by the smaller solid line adrenal which represents the atrophic adrenal, and the dotted portion represents the adrenal as

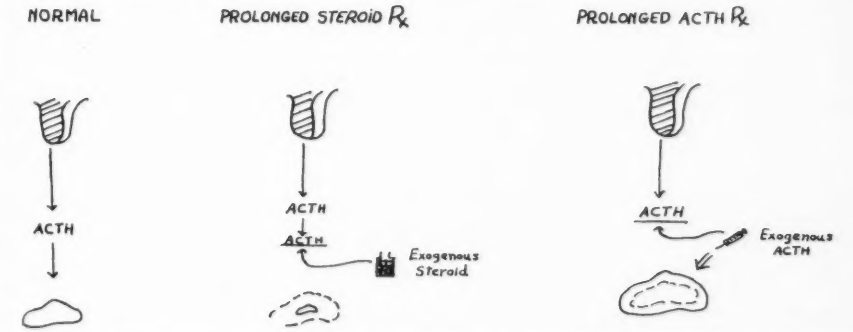


Fig. III. Effects of exogenous steroid or ACTH on endogenous pituitary ACTH secretion.

it was in the original state.

Another factor, not always generally appreciated, is that the administration of exogenous ACTH is **also** capable of inhibiting the pituitary elaboration of ACTH while stimulating the adrenal to greater activity and size. This is demonstrated in Fig. III with the more solid line representing the stimulated adrenal and the dotted line representing the original size. The significance of this soon will become more apparent. However, needless to say, this may have a favorable effect on the recovery mechanisms of adrenal function with cessation of ACTH therapy and therefore should be kept in mind.

where the endogenous ACTH stimulus is deficient or absent, exogenous ACTH will produce a sluggish but definite adrenal response in most instances, though it may take several days of stimulation. On the other hand, in the presence of Cushing's syndrome where the adrenal is already overactive due to adrenal hyperplasia, the administration of ACTH will produce a further hyperreactive response.

We already know that the administration of exogenous cortisol will inhibit pituitary ACTH release. Such therapy therefore can lead to adrenal atrophy, but this usually requires large dosage for several days. However, in an

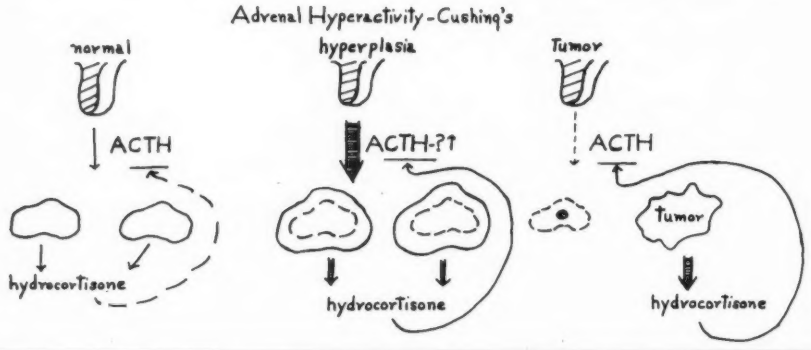


Fig. IV. Interaction in Cushing's syndrome between pituitary and hyperplastic adrenals and adrenal tumor.

Thus, when exogenous ACTH is administered to normal individuals, the adrenal may be stimulated to greater function, and this increased function may be measured by blood 17-hydroxycorticosteroid response. If an infusion of 25 milligrams of ACTH is given to normal individuals over a four-hour period, there will be a rise from a mean control value of plasma 17-hydroxycorticosteroids of 16 micrograms percent to a value of approximately 40 micrograms percent. This represents a normal adrenal response to exogenous ACTH. If the individual has primary Addison's disease, such exogenous ACTH stimulation will cause no rise in the blood 17-hydroxycorticosteroid levels. In hypopituitarism, acute study[7], the intravenous administration of cortisol has shown evidence of adrenal suppression by interference with ACTH release by the pituitary in a period of hours.

These changes and effects from exogenous steroid therapy are sometimes duplicated in certain pathological states, for example Cushing's syndrome. In the presence of an adrenal tumor producing Cushing's syndrome (which tumor produces hydrocortisone in excess and is autonomous of pituitary control), excessively produced hydrocortisone is capable of inhibiting the pituitary elaboration of ACTH (Fig. IV). This ACTH inhibition then removes the normal pituitary stimulus to the normal contralateral adrenal.

and therefore this adrenal becomes atrophic. Here is an example of excessive amounts of hydrocortisone produced endogenously which is then capable of inhibiting pituitary ACTH and causing adrenal atrophy on the other side. In Cushing's syndrome due to hyperplasia, no actual increase in pituitary elaboration of ACTH has been demonstrated, but it is postulated that a substance may be produced by the pituitary that is capable of potentiating normal amounts of ACTH[8]. This then stimulates the adrenals to hyperplasia and they enlarge.

Prolonged Steroid Therapy

With the above serving as background material, it is well now to go into some of the many animal experiments that were done in the past which have helped to focus attention on the possible serious effects that exogenous adrenal steroid therapy may have upon the human organism. Ingle and co-authors in 1937[1] demonstrated that the administration of large quantities of exogenous adrenocortical extract to a normal rat was capable of producing atrophy of the adrenal cortex. They further showed that this atrophy could be prevented by the simultaneous administration of a small quantity of impure adrenocorticotropin.

Winter and co-authors in 1950[9] admininstered cortisone acetate to rats. After 10 days of this therapy, they noted a 40% loss of adrenal weight in these animals and in 6 weeks note that this loss amounted to over 50% of their original adrenal weight as compared to controls. On discontinuation of therapy, recovery of the adrenal atrophy was detectable within 4 days, was well advanced within 10 days, and was unquestionably complete in 45 days after cessation of therapy. Thus far, it is apparent that the administration of adrenocortical material is capable of producing adrenal atrophy in animals; concomitant administration of an ACTH substance with this adrenal cortical extract is capable of neutralizing the atrophic effect; and finally on discontinuation of suppressive therapy, it is apparent that this adrenal atrophy is reversible though taking a variable period of time for recovery.

Lewis and co-authors in 1950[10] then demonstrated that the atrophic adrenal effect of cortisone was via its inhibition of the pituitary elaboration of ACTH. They administered cortisone acetate to intact rats and obtained adrenal atrophy. Then they gave the same type of therapy to hypophysectomized rats who were maintained on exogenous ACTH and in these instances there was **no** effect on the adrenal size. Thus, the cortisone did not inhibit the exogenous ACTH. One can infer from this study that cortisone induces adrenal atrophy by inhibiting the pituitary elaboration of ACTH directly.

Collins and Olsen in 1954[11] administered cortisone or hydrocortisone to dogs and were able to suppress adrenal function. This suppression was maintained for 2 weeks after cessation of the therapy. When they administered ACTH simultaneously with the cortisone or hydrocortisone, the adrenal suppression was prevented, and the adrenal responsiveness was maintained.

Sydnor in 1955[12], in a study on adrenalectomized rats, demonstrated that intravenous hydrocortisone was capable of blocking ACTH release by the pituitary in the stress adrenalectomized rats within one or two minutes. Therefore, one can see how rapidly the steroid may work in suppressing pituitary release of ACTH.

Progressing from animal to human studies, Sokoloff et al in 1951[13] examined the adrenals of patients with various rheumatic diseases. Some of these patients had received variable doses of ACTH or cortisone. These authors noted that in those patients who had received ACTH hypertrophy of the adrenal cortex was demonstrable pathologically, and those patients who received cortisone exhibited an atrophy of the adrenal cortex.

Engelman et al in 1953[14] studied a series of patients who had been on long term adrenal steroid therapy and attempted to see how long it would take to stimulate the suppressed adrenal to normal function by the administration of ACTH. On this basis they found that by the 6th day of stimulation, 100% of their patients responded normally to ACTH. The authors concluded from this that adreno-

cortical function may be suppressed by the long term administration of cortisone, even in maintenance doses. However, and this is most important, this adrenocortical suppression is reversible.

Larzelere and co-authors[15] studied 22 patients who had been on varying doses of cortisone, hydrocortisone or prednisone for periods up to five years. They administered intravenous ACTH to these patients to determine their adrenal responsiveness. All of their patients responded to ACTH, but 16 of the 22 demonstrated a delayed response in that it took three to five days. They found no apparent correlation in this series between the daily dose of the suppressive steroid, the duration of therapy, and the adrenal response to ACTH. Quoting these authors: "since all of these patients ultimately demonstrated adrenal responsiveness, even though they had been receiving cortisone continuously for as long as 6 years, there would appear to be no need for the intermittent withdrawal of cortisone to protect against irreversible adrenal atrophy; nor should intermittent courses of corticotropin be necessary during long term therapy with cortisone."

Comparative Steroid Potencies

Having briefly presented clinical studies of the effects of prolonged steroid therapy on adrenal function, it is fitting to compare the potencies of the various adrenocortical steroids that are currently being utilized in the therapy of various clinical disorders. Cortisone, which was one of the earliest pure steroids used, has been given in pharmacologic dosage; for example, 100 to 300 milligrams per day, in the treatment of various medical diseases. On the other hand, the use of steroid in endocrine disorders is usually limited to physiologic dosage which is a much lower range. Hydrocortisone, which is approximately one and one half times more potent than cortisone, is used in lesser dosage. More recently, the advent of steroids as prednisone and prednisolone, which are at least 5 times as potent as cortisone, makes them effective in much lower dosage, e.g., 10 to 30 milligrams per day. Their efficacy is judged by their ability to inhibit the pituitary elaboration of ACTH as well as by their clinical effects.

ACTH, which acts in a different fashion, may be used intravenously in dosage of 25 milligrams over a 6 to 8 hour period; intramuscularly, 25 milligrams every 6 hours; or as a repository in single dosage, once per day, or more preferably in dosage of approximately 40 units every 8 to 12 hours. The comparative strengths of these various compounds must be appreciated as must their ability to induce adrenal atrophy.

Christy et al[16,17] administered prednisone or cortisone and then noted the effect on the blood 17-hydroxycorticosteroid response to ACTH. They found that the minimum amount of prednisone needed to suppress the adrenal response was 20 milligrams per day for 7 days. On the other hand, cortisone in doses of 100 milligrams or more for 7 to 10 days did not alter the ACTH response. The authors concluded from this that prednisone appeared 5 times as potent as cortisone in suppressing endogenous ACTH release. In further studies, they stated that treatment with ACTH may accelerate the return of post steroid adrenocortical re-activity from the sub-normal to normal state. They note, however, that the effect of ACTH in improving adrenal responsiveness should not be interpreted as implying a normal capacity of the pituitary to react to sustained stressful stimulus.

Counteraction of Adrenal Suppression

In view of all that has been said, several authors have attempted to evaluate varying regimes that might tend to minimize the markedly adrenal suppressive effect of adrenocortical steroid therapy.

Birke et al[18] did such a study. One of their groups was treated with 40 milligrams of hydrocortisone per day for 6 days, and on the 7th day 5 to 20 units of a long-acting ACTH preparation was given. Then the course of hydrocortisone was repeated. This was compared to other groups given different regimes of therapy. In this particular group, the patients showed almost complete adrenal suppression after the 6th day of hydrocortisone therapy, and the one dose of long-acting ACTH apparently restimu-

lated these adrenals to a certain degree. However, their data did not appear as conclusive as they apparently interpreted it to be. In any event, they advocated intermittent ACTH therapy to those patients who are on long term steroid suppression, utilizing ACTH every 7 to 10 days, and withholding the adrenal steroid on that day.

concluded that the concurrent administration of repository corticotropin at weekly intervals in very high dosage will usually prevent the development of adrenal unresponsiveness. Once again the data did not seem very convincing.

The upper portion of Fig. V demonstrates that on exogenous steroid therapy the pituitary elaboration of ACTH

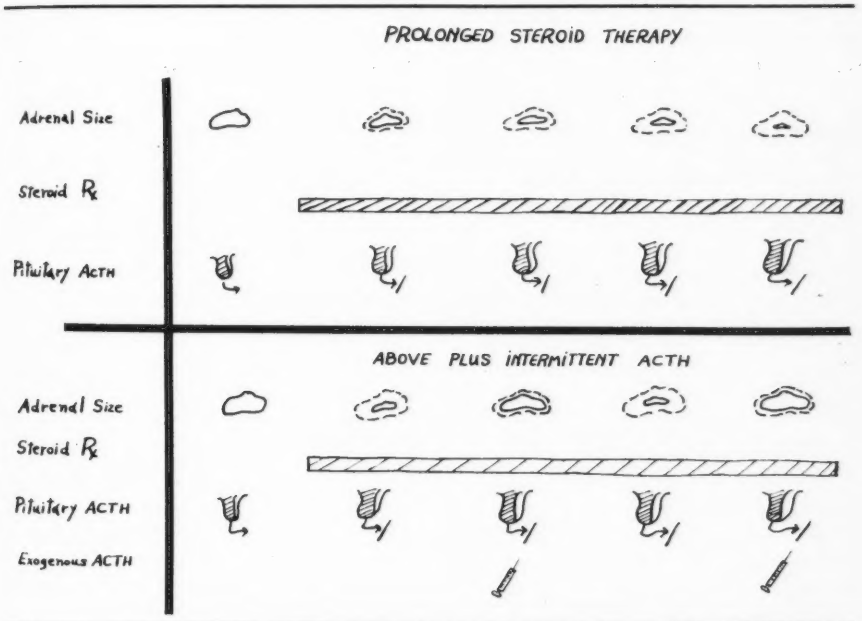


Fig. V. Effects of prolonged steroid therapy with and without exogenous ACTH on the adrenals and pituitary.

Young et al[19] stated that since there was some evidence of adrenocortical activity appearing spontaneously in some patients approximately 3 to 4 days after suppressive adrenal steroid therapy is withdrawn, it would appear that inhibition of corticotropin production by the high blood cortical levels induced by this endogenous therapy is usually rather transient. They felt that in the presence of stress, the limiting factor in the production of hydrocortisone by the adrenal may well be the adrenocortical atrophy rather than the suppression of corticotropin by the exogenous steroid therapy. On this basis, they performed a study and

is suppressed, and concomitantly there is a decrease in the size of the adrenal gland with it becoming progressively more and more atrophic. The bottom portion shows the same steroid therapy; but with intermittent ACTH administration. On this regime it is apparent that there is an intermittent stimulation of a suppressed adrenal, but at the same time there is a continuous suppression of the pituitary elaboration of ACTH.

Since it is known that exogenous adrenal steroid therapy will induce adrenal atrophy (which atrophy is reversible), and furthermore, since it is known that such therapy inhibits the endogenous

release of ACTH as does the administration of exogenous ACTH, there appears to be no advantage in the intermittent administration of exogenous ACTH. An off and on stimulation of a suppressed adrenal is produced with intermittent ACTH, and there is apparently no question that this does place the adrenal in a more responsive state. However, as soon as one gives exogenous adrenal steroid again, after having administered the intermittent ACTH injection, the adrenal is once again suppressed. The pituitary is maintained in complete suppression all the time, so that even with intermittent ACTH stimulation the **abrupt** withdrawal of the adrenal steroid and/or ACTH may leave the adrenal in a more responsive state, but there is no stimulus from the pituitary to maintain it in this responsive state. Furthermore, there is no apparent correlation as to the degree of adrenal suppression with the amount of exogenous steroid administered or how long the steroid is given. Therefore, one cannot tell how an individual case will respond.

Furthermore, one basic physiologic fact is still apparent — the adrenal seemingly cannot be irreversibly atrophied by steroid therapy. Therefore, it seems much more logical when an individual maintained on suppressive adrenal steroid therapy is a candidate for withdrawal to taper off such dosage and combine this with ACTH by injection or infusion, without subjecting the patient to intermittent ACTH for whatever dubious result it may give.

In conclusion, therefore, neither do we know the final answer nor do we know the best way to evaluate a patient's ability to respond to stress such as operative trauma after cessation of steroid therapy. There is too much variation from patient to patient. However, the best therapy is prophylactic therapy. This implies first the use of adrenal steroids only in selected cases where it is absolutely essential. Further, one must consider the following at the time such therapy is withdrawn: (1) ACTH must be given concomitantly, and (2) the adrenal steroid must be stopped first while the ACTH is maintained and then tapered off. The course of the patient during this procedure is to be followed with urinary 17-hydroxycorticosteroids to determine when his adrenal is functioning at a normal level spontaneously again.

Summary

Due to basic pituitary-adrenal physiologic interrrelationships, the administration of exogenous adrenal steroid as cortisol is capable of inducing reversible adrenal atrophy. Abrupt cessation of such therapy may produce an acute adrenal insufficiency. After discussion of animal and human studies pertinent to this complication, it would appear most prudent to taper such steroid therapy at its termination and include a course of ACTH stimulation which should also be tapered. Since adrenal atrophy is not predictable regardless of duration or dose of steroids administered, the above procedure would be utilized best routinely.

BIBLIOGRAPHY

1. Ingle, D. J. and Kendall, E. C.: Atrophy of the adrenal cortex of the rat produced by the administration of large amounts of cortin. Science, *86*:245, 1937.
2. Soffer, L. J.: Diseases of the endocrine glands, p. 369, 1951. Lea & Febiger, Philadelphia.
3. Salassa, R. M., Bennett, W. A., Keating, F. R., Jr. and Sprague, R. G.: Postoperative adrenal cortical insufficiency. J. Am. Med. Assoc., *152*:1509, 1953.
4. Ingle, D. J.: The role of the adrenal cortex in homeostasis. J. Endocrinol., *8*:23, 1952.
5. Engel, F. L.: The adrenal cortex and the metabolic response to stress. J. Clin. Endocrinol. and Metab., *13*:1555, 1953.
6. Sayers, G.: The adrenal cortex and homeostasis. Physiol. Rev., *30*:244, 1950.
7. Jailer, J. W. and Wallace, E. Z.: The effect of intravenously administered hydrocortisone on the urinary 17-ketosteroids in patients with adrenal virilism. Ann. N. Y. Acad. Sci., *61*:442, 1955.
8. Jailor, J. W. Longson, D. and Christy, N. P.: Cushing's syndrome—An adrenal or pituitary disease? J. Clin. Endocrinol. and Metab., *16*:1276, 1956.
9. Winter, C. A., Silber, R. H. and Stoerk, H. C.: Production of reversible hyperadrenocortinism in rates by prolonged administration of cortisone. Endocrinol., *47*:60, 1950.
10. Lewis, R. A., Rosenberg, E. and Wilkins, L.: The effects of 17-hydroxy-11-dehydrocorticosterone upon the adrenals of normal and of hypophysectomized rats maintained with adrenocorticotropin. Endocrinol., *47*:414, 1950.

11. Collins, E. J. and Olsen, K. J.: Inhibition of steroid-induced adrenal hypofunction. Proc. Soc. Exper. Biol. and Med., *87*:76, 1954.

12. Sydnor, K. L.: Blood ACTH in the stressed adrenalectomized rat after intravenous injection of hydrocortisone. Endocrinol., *56*:204, 1955.

13. Sokoloff, L., Sharpe, J. T. and Kaufman, E. H.: The effect of ACTH and cortisone on the human adrenal gland. Am. J. Path., *27*:706, 1951.

14. Engelman, E. P., Krupp, M. S., Johnson, H. P., Welsh, J. E., Wrenn, H. T., and King, W. R.: Adrenocortical function during continuous long-term therapy with cortisone. Arch. Int. Med., *91*:1, 1953.

15. Larzelere, R. G., Jr., Barthold, E. A., Willett, F. M., Feichtmeir, T. V., Wilson, L. and Engleman, E. P.: Adrencortical function in long-term treatment with corticoids. A.M.A. Arch. Int. Med., *99*:888, 1957.

16. Christy, N. P., Wallace, E. Z. and Jailer, J. W.: The effect of meticorten on the inhibition of ACTH in the human subject. Proceedings of the first International Conference on The Clinical and Metabolic Effects of Prednisone and Prednisolone, pp. 249-255, May 31 and June 1, 1955. New York, N. Y.

17. Christy, N. P., Wallace, E. Z. and Jailer, J. W.: Comparative effects of prednisone and of cortisone in suppressing the response of the adrenal cortex to exogenous adrencorticotropin. J. Clin. Endocrinol. and Metab., *16*: 1059, 1956.

18. Birke, G., Domeij, B., Borje, O. and Plantin, L.: Measures for the avoidance of adrenal atrophy in prolonged corticosteroid therapy. Acta. Med. Scand., *155*:245, 1956.

19. Young, I. I., DeFilippis, V., Meyer, F. L. and Wolfson, W. Q.: Maintenance of adrenal cortical responsiveness during prolonged corticoid therapy. A.M.A. Arch. Int. Med., *100*:1, 1957.

THE ORALLY ACTIVE HYPOGLYCEMIC AGENTS *

PIERO P. FOA, M.D., Ph.D. and GIORGIO GALANSINO*****

The search for orally active antidiabetic agents and insulin substitutes predates the discovery of insulin itself. Innumerable substances including vasodilators, antihistaminics, salicylates, anticoagulants, oral insulin preparations, plant extracts and plant hormones have been tried[10-12], but only a few have had more than fleeting success. Some of the better known substances are included in Table 1; the chemical characteristics and mode of action of these and of other compounds have been discussed in recent publications[1-9] and will be reviewed only briefly.

I. **Guanidine Derivatives.** Synthalin A and B were among the earliest hypoglycemic drugs to receive fairly extensive clinical trials[10]. However, their success was short-lived, primarily because they produced frequent side reactions and occasional necrotic lesions of liver and pancreas in animals, partly because their usefulness was limited to the mild or moderately severe adult diabetics, and perhaps also because insulin had been discovered only a few years before. Other guanidine derivatives are now under investigation[1-9, 13, 14]. The best known among them is a phenethyldiguanide called DBI, PEDG or PEBG which causes marked hypoglycemia accompanied by depletion of liver glycogen, evidence of A-cell degranulation, and changes in the adrenal cortex and kidney[15]. PEDG is believed to inhibit gluconeogenesis and cellular respiration and to stimulate anaerobic glycolysis[16-18]. Contrary to other hypoglycemic agents, PEDG is insulin independent and is therefore effective in alloxan-diabetic animals[19] and in patients with growth-onset or juvenile diabetes[20, 21]. Unfortunately, the clinical usefulness of PEDG is limited by the severity and frequency of side effects[20]. (Footnote 1.)

II. **Hypoglycin A and B.** The ingestion of unripe fruits of *Blighia sapida*, the Ackee tree of the West Indies or Ishin tree of Africa, causes severe "vomiting sickness", hypoglycemia, convulsions, coma and sometimes death with pulmonary edema and damage to the liver, kidney, gastric mucosa and lymphatic tissue[22]. The active principles of *Blighia sapida* are Hypoglycin A, a 7-carbon cyclic amino acid, and Hypoglycin B, a dipeptide of Hypoglycin A and glutamic acid [1-9, 23, 24]. These substances do not require the presence of the adrenals or the pancreas[25], and have metabolic effects different from those of insulin[26]. Their clinical usefulness has not been investigated because of their marked emetic and toxic effects.

III. **The Sulfonylureas A. General.** In 1942 Janbon and collaborators[27] accidentally discovered the hypoglycemic effects of p-aminobenzene-sulfamide-isopropylthiodiazol (2254-RP, VK-57, or IPTD), while screening several compounds for their antibacterial properties. Although Loubatieres understood the importance of this observation[28], interest in the problem did not become widespread until after the publication of experimental and clinical results obtained with carbutamide (BZ55). Soon other substances with hypoglycemic activity were synthesized, including tolbutamide or D860, chlorpropamide or P607, metahexamide or C29880 and many others[6, 28-32].

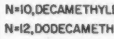
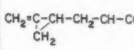
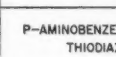
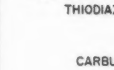
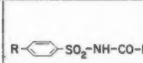
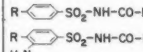
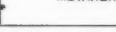
* Aided by Grant A-522, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

** Professor, Department of Physiology and Pharmacology, The Chicago Medical School.

*** Trainee, Diabetes Training Program 2A-5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

1. Recent evidence indicates that concentrations of DBI lower than those used in previous studies and more similar to those obtained *in vivo* following administration of therapeutic doses, may stimulate the utilization of glucose via the shunt pathway[163-164]. In small doses and in conjunction with insulin, DBI may deserve careful clinical trial in selected cases of brittle or juvenile diabetes. The clinical usefulness of this drug has been evaluated recently.

ORALLY ACTIVE HYPOGLYCEMIC AGENTS

TYPE	GENERAL FORMULA	R	COMPOUND
DIGUANIDES	$H_2N-C(=NH)-NH-(CH_2)_N-NH-C(=NH)-NH_2$ $R-NH-C(=NH)-NH-C(=NH)-NH_2$	 $CH_3-(CH_2)_4-$ $(CH_3)_2-(CH_2)_3-$	N=10, DECAMETHYLENE-DIGUANIDINE, SYNTHALIN A N=12, DODECAMETHYLENE-DIGUANIDINE, SYNTHALIN B PHENETHYLDIGUANIDE, PEDG, DBI AMYL-DIGUANIDE ISOAMYL-DIGUANIDE
7C-∝-AMINO ACIDS		-OH -GLUTAMIC ACID	∅-(METHYLENE-CYCLOPROPYL)-L-∝ AMINOPROPIONIC ACID, HYPOGLYCIN A HYPOGLYCIN B
SULFONYLAMIDES		 	P-AMINOBENZENE-SULFAMIDO-ISOPROPYL-THIODIAZOL, 2254 RP, IPTD CARBUTAMIDE, BZ 55
		R / R_1	
SULFONYLUREAS	 	-CL / $-C_3H_7$ $-CH_3$ / $-C_4H_9$ $-CH_3$ / 	CHLORPROPAMIDE, P607 TOLBUTAMIDE, D860 METAHEXAMIDE, C29880, WP-40

B. Chemistry. The chemical composition of the best known sulfonylureas is given in Table 1. Methods for their estimation in blood and urine are available[8, 9, 33-37]. For added information on the chemistry of these and other compounds, and on the relationship of chemical structure to hypoglycemic potency, the reader is referred to other studies[1-9].

C. Pharmacology, Absorption, Fate, Excretion and Dosage. The sulfonylureas are absorbed rapidly and excreted, metabolized or inactivated in different ways and at variable speeds[1-9, 33], so that effective plasma concentrations are maintained for different lengths of time[38]. For example, the "half-life" of carbutamide and chlorpropamide in the blood is 30 to 40 hours, while that of tolbutamide is about 4 hours[39-41]. This variability in the rate of disposal, coupled with the intrinsic potency and toxicity of the drugs, determines their average effective dose and side effects[1-9, 39-47]. Large doses of these drugs may injure liver, kidney, pancreas, adrenals and other organs[1-9, 48] and impair fetal development[49] in the experimental animal, but in therapeutic doses their toxicity is gratifyingly low. The most frequent side effects are anorexia, nausea, vomiting, gastro-intestinal discomfort, skin eruptions, leucopenia, muscular weakness and fever[1-9]. In a series of 9163 cases of diabetes treated with tolbutamide; the incidence of side reaction was only 3.2% (292 cases), and only in 173 patients (1.9%) were these effects sufficiently severe to require interruption or discontinuation of therapy[50]. The rate of side reactions in approximately 2500 cases treated with metahexamide seems to be higher (about 6.5%, with several cases of jaundice), but this series includes a number of patients treated with excessively large doses[9]. (Footnote 2.) Ataxia, severe neuromuscular reactions[51-52] and rare cases of death have been reported[53]. In some cases, death may have been due to withdrawal of insulin rather than the drug itself[54]. Hypoglycemic reactions may occur, but, in general, they are not as severe as those caused by an overdose of insulin. (Footnote 3.)

Mode of Action. 1) Inhibition of anterior pituitary, adrenocortical or thyroid hormone secretions or action or stimula-

2. Recently, metahexamide was withdrawn from clinical trial for excessive liver toxicity.

3. Severe hypoglycemic shock due to chlorpropamide was reported recently[166].

tion of their destruction. This mode of action has been ruled out by the observation that hypophysectomy and thyroidectomy do not decrease and adrenalectomy greatly enhances the hypoglycemic response to sulfonylurea administration[55-58].

2) Inhibition of glucagon secretion or action, or enhancement of glucagon destruction. Early observers reported that the hypoglycemic drugs cause extensive damage to the A-cells of the islets of Langerhans, and suggested that their effect was due to a decreased secretion of glucagon. These results have been criticized[59] and have not been confirmed by subsequent investigation[38,60,61]. Although occasionally a decrease in stainability of the A-cells has been observed in treated animals, this may reflect the acute glucagon discharge believed to occur as a homeostatic response to hypoglycemia [62], and may be the result rather than the cause of hypoglycemia[63]. Prolonged treatment with tolbutamide does not affect the glucagon content of the pancreas[64]. Some investigators have reported that tolbutamide treatment interferes with the action of glucagon *in vivo*[59,65] and *in vitro*[66], but other investigators have not confirmed these results[60,67,68]. Moreover, the degradation of glucagon is unaffected by tolbutamide treatment[68,69], suggesting that glucagon is not a significant factor in sulfonylurea hypoglycemia.

3) Inhibition of liver glucose production. Liver glycogenolysis requires three steps: phosphorylation of glycogen to glucose-1-phosphate, conversion of glucose-1-phosphate to glucose-6-phosphate and hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate. The first reaction is catalyzed by phosphorylase, the synthesis of which is promoted by glucagon. The second reaction is catalyzed by phosphoglucomutase and the third by glucose-6-phosphatase. There is no evidence that the sulfonylureas depress liver phosphorylase and phosphoglucomutase activity[70]. The results of glucose-6-phosphatase measurements have not been uniform: one group of investigators[71] found that oral carbutamide does not depress glucose-6-phosphatase activity in liver homogenates of normal and alloxan-diabetic rats; other workers found that oral tolbutamide depresses the enzyme activity in the liver of normal rats[72,73] and restores to normal the activity increased by alloxan treatment[74]. According to some investigators[75,76], tolbutamide added *in vitro* to rat liver homogenates, in concentrations similar to those which must be reached in plasma to obtain hypoglycemia, causes slight, but reproducible inhibition of glucose-6-phosphatase activity. According to others[70,77], this phenomenon can be observed only when large concentrations of the drug are used. Several DPN-dependent liver dehydrogenases may also be depressed by the sulfonylureas[78]. More convincing evidence of a decreased release of glucose from the liver has been obtained from studies of the rate of disappearance of C^{14}-labeled glucose from plasma after intravenous administration[79-81], from measurements of liver glucose output by means of hepatic and portal vein catheterization[82,83], and from the inhibition of fructose or galactose induced hyperglycemia[84-86]. As expected, this decrease in liver glucose output is accompanied by an increase of liver glycogen[86-91], and of the incorporation of C^{14}-labeled glucose into liver glycogen[92,93].

Although these experiments suggest that the liver is a major site of action of the sulfonylureas, the presence of the liver is not essential; tolbutamide is just as effective in hepatectomized dogs maintained on constant glucose infusion as in normal dogs[94,95]. On the other hand, sulfonylureas are completely inactive in totally depancreatized or severely alloxan-diabetic animals[28,55,56,96-102], a fact suggesting that hypoglycemia may be the result of an insulin sparing action or a stimulation of insulin release.

4) Insulin sparing action. Under normal conditions, insulin is destroyed by insulinase systems present in various tissues. Tolbutamide and carbutamide, administered *in vivo*[98,99] or added *in vitro*[103], inhibit liver insulinase activity. However, this effect does not appear sufficient to alter the over-all insulin degradation processes in the intact organism [68,69,104], and may not be specific[105]. It is possible that the increased activity of insulin in the presence of sulfonylureas,

observed *in vitro*[106] and *in vivo*[107-110], may be the result of additive or potentiating rather than insulin sparing effects.

5) Stimulation of insulin release. The following experiments and clinical evidence suggest that the sulfonylureas may stimulate insulin release from the pancreas: a) drug administration is followed by marked degranulation[100,102,111], increased mitotic activity[112] and hyperplasia[61] of the B-cells; b) tolbutamide does not cause hypoglycemia when the B-cells have been completely degranulated and possibly pre-emptied of their insulin content by previous treatment, such as in partially depancreatized animals treated with adrenal steroids and in animals with metahypophyseal diabetes[113]; c) pre-treatment with tolbutamide lessens the initial alloxan hypoglycemia believed to be caused by the release of insulin from the destroyed B-cells[114]; d) degranulation of the B-cells is accompanied by an increase in the insulin activity of pancreatic, portal and systemic blood, as indicated by cross-circulation experiments[28,115] or by means of the rat diaphragm method in some[64,112,116-118], although not in other experiments[119,120]; e) small quantities of sulfonylureas injected into the pancreatic artery cause hypoglycemia, according to some investigators[28,121,122], but inconclusive or negative results according to others[107,123,124]; f) the injection of a new sulfonylurea derivative causes a decrease in the deposition of S^{35}-labeled cystine in the pancreas of rats[125,126]. In accordance with the hypothesis of insulin release, there is an apparent relationship between hypoglycemic potency of the drugs and pancreatic reserve of the individual. Thus, hypoglycemia is more marked in normal than in diabetic subjects[127] and in diabetic subjects with high plasma insulin activity than in patients with low activity or none at all[120].

Although based on strong experimental and clinical evidence, insulin release by the pancreas cannot be accepted as the sole explanation for the hypoglycemia unless it can be shown that other metabolic effects of insulin are also shared by the drugs. Results of experiments designed to study this point have not been uniform. For example, some investigators reported that treatment with sulfonylureas improves the carbohydrate tolerance of normal animals and diabetic subjects, while other investigators could find no significant changes[1-9]. The action of sulfonylureas on muscle glycogen is also uncertain, as it has been reported that its synthesis is stimulated[93,128], inhibited[89] or unaffected[82,87,129] by tolbutamide administered *in vivo*. Most investigators (with at least two exceptions [130,131]) believe that, contrary to insulin, carbutamide and tolbutamide do not stimulate glucose uptake by the isolated rat diaphragm[105,132] or by the isolated frog muscle[133]. The effect of sulfonylureas on glucose utilization by peripheral tissues *in vivo* is also uncertain; it has been reported that tolbutamide, like insulin, increases in peripheral glucose utilization in normal dogs, as indicated by artero-venous glucose difference[134,135], and that in diabetic patients tolbutamide, like insulin, lowers the minimum concentration of blood glucose at which utilization begins[136]. However, other workers[84,137-139] could find no evidence of increased peripheral utilization of glucose.

Unlike insulin, the sulfonylureas promote glycogen deposition in the liver[87,89,91,92], and do not increase but decrease blood lactate and pyruvate[123,124,140-142]. Like insulin, tolbutamide decreases the concentration of plasma amino acids[143], non-esterified fatty acids[144], and ketone bodies[90], but, unlike insulin, it does not affect the disappearance of D-xylose and L-arabinose from the blood[145]. Uncertain is the effect of sulfonylureas on serum inorganic phosphate which was found decreased in normal and in diabetic subjects by some investigators[116,146], but unchanged by others[84,147], and on plasma potassium which also was found decreased by some investigators [116,148] and unchanged by others[123,124,140,141,147,149]. The reasons for these discrepancies are not clear. In some cases the pancreatropic effect may have been too small to be detected; in other cases it may have occurred at a time when the insulin content of the pancreas was already depleted[90]. However, other explanations are also possible: perhaps the drugs act on the liver and other tissues

as well as on the pancreas or perhaps insulin, secreted directly into the portal system as a result of normal pancreatic function or pancreatic stimulation, acts differently from insulin injected peripherally. Experiments designed to study these possibilities also have given contradictory results[9]. Several groups of workers studied the effect of tolbutamide and of insulin injected into the portal or into the femoral vein on the rate of disappearance from the plasma of C^{14}-labeled glucose. One group[83] concluded that tolbutamide causes hypoglycemia by blocking the release of glucose from the liver, while insulin, whether injected into the portal or into the femoral vein, acts primarily by increasing peripheral glucose utilization. The second group of workers[80] confirmed the observation with tolbutamide and insulin injected intravenously, but, in addition, found that when insulin is injected subcutaneously and is slowly absorbed, it acts like tolbutamide, causing a suppression of glucose entry into the blood without affecting its removal. Still another group of workers[150] reported that, at comparable levels of hypoglycemia, injections of insulin into the portal vein are followed by smaller increases in peripheral glucose utilization than injections into a peripheral vein and suggest that the metabolic effects of insulin may vary depending upon the route of administration. Unfortunately, these conclusions are based on the measurement of A-V differences without simultaneous measurements of blood flow.

There are indications that when small doses of insulin are injected into the portal vein a relatively large fraction of it is destroyed or captured by the liver and that little or no insulin appears in the general circulation. With increasing doses, increasing amounts of insulin would escape the liver and become available for peripheral action[9]. These observations suggest that differences between the findings of various investigators may be the result of dose and rate of administration rather than of intrinsic qualitative differences in mode of action.

Experiments performed in this laboratory[123,124,140,141] did not disclose significant qualitative differences between the effects of insulin injected into the portal or into the femoral vein of anesthetized dogs: in all cases insulin caused an increase in the concentration of blood pyruvate and lactate and a marked decrease in plasma potassium. On the other hand, the intravenous administration of carbutamide, tolbutamide, chlorpropamide and metahexamide caused a decrease not only in blood glucose, but also in blood pyruvate and lactate and only an occasional, but small decrease in plasma potassium. These results do not contradict the strong evidence for a pancreatropic action of the sulfonylureas, but suggest that the drugs may act in other ways also.

IV. Conclusions. The experimental evidence reviewed in this paper suggests that sulfonylureas act by suppressing liver glucose production and that this action is possible only when insulin is injected or released in "permissive" amounts. This hypothesis would explain why the drugs cause hypoglycemia in the absence of the liver when glycogenolysis is absent, insulin secretion normal and insulin destruction normal or reduced, and why they are not effective in the absence of functioning pancreatic tissue or of exogenous insulin when glycogenolysis and gluconeogenesis proceed at an overwhelming rate. Thus, carbutamide and tolbutamide may exert their hypoglycemic effect only in normal, partially alloxanized or partially depancreatized animals, or in mild diabetic patients where insulin may be provided by the stimulation of the B-cells in quantities sufficient to make the liver action of the drugs possible. Essentially the same conclusion was reached recently by other investigators[148].

If this hypothesis is correct, prolonged therapy with sulfonylurea-like drugs should be attempted with caution, for the suppression of hepatic glucose production may be a sign of injury and, although continued stimulation of the B-cells may result in hypertrophy and increased insulin production[61,151–154], it may lead also to functional insufficiency. That this may happen is suggested by the inability of the pancreas to respond to further stimulation[117], by the decreased glucose tolerance[152] and by the

gradual increase of fasting blood glucose to diabetic levels[155], observed in chronically treated rats.

In addition to the possibility of functional and structural damage, harm may be done by withholding insulin therapy; the problem of diabetic complications has not been solved, as a disturbingly large number of patients still suffer from neuritis, retinitis, lens opacities, glomerular and other vascular lesions[156]. Are these lesions related to the alterations in carbohydrate metabolism, to the disturbed lipid and lipoprotein composition of the serum, or to other unknown factors? We do not know the answers to these questions, but we do know that the major metabolic defects of diabetes can be corrected to a great extent with insulin. It is doubtful whether the same goals can be reached with the sulfonylureas which do not appear to stimulate glucose utilization directly and may[144,157] or may not[158] correct the blood lipid aberrations. For these reasons it may be wise to give the patients the benefit of the doubt and use oral therapy only as an adjuvant to insulin[39,159-162] or in those patients who cannot be treated with diet alone, who refuse insulin or for whom the use of insulin is made hazardous by physical or mental handicaps.

REFERENCES

1. Foa, P. P.: Chicago Medical School Quart., *17*:145, 1956.
2. Atti lo Simposio Nazionale sul Diabete, Settimana Medica, Firenze, 1956.
3. The Effects of the Sulfonylureas and Related Compounds in Experimental and Clinical Diabetes. Ann. N. Y. Acad. Sci., *71*:1, 1957.
4. Third Lilly Conference on Carbutamide. Diabetes, *6*:1, 1957.
5. Symposium, Deut. Med. Wschr., *82*:1513, 1957.
6. Gourley, D. R. H.: Proc. Soc. Exp. Biol. Med., *99*:69, 1958.
7. Larizza, P., Grignani, F. and Brunetti, P.: Minerva Medica., *49*:631, 1958.
8. Conference on Chlorpropamide and Diabetes Mellitus. Ann. N. Y. Acad. Sci., *74*:407, 1959.
9. Conference on Insulin and the Oral Hypoglycemic Agents. Metabolism (in press).
10. Goldner, M. G.: A.M.A., Arch. Int. Med., *102*:830, 1958.
11. Perez, C. and Paris, R.: Ann. Pharm. Franc., *16*:86, 1958.
12. Garcia-Blanco, J. and Solsano, M.: Rev. Espan. Fisiol., *9*:31, 1953.
13. Williams, R. H., Tanner, D. C. and Odell, W. D.: Diabetes, *7*:87, 1958.
14. Odell, W. D., Tanner, D. C., Steiner, D. F. and Williams, R. H.: A.M.A. Arch. Int. Med., *102*:520, 1958.
15. Creutzfeldt, W. and Moench, A.: Endokrinologie, *36*:167, 1958.
16. Tyberghein, J. M. and Williams, R. H.: Proc. Soc. Exp. Biol. Med., *96*:29, 1957.
17. Williams, R. H., Tyberghein, J. M., Hyde, P. M. and Nielsen, R. L.: Metabolism, *6*:311, 1957.
18. Wick, A. N., Larson, E. R. and Serif, G. S.: J. Biol. Chem., *233*:296, 1958.
19. Ungar, G., Freedman, L. and Shapiro, S. L.: Proc. Soc. Exp. Biol. Med., *95*:190, 1957.
20. Krall, L. P. and Camerini-Davalos, R.: A.M. A. Arch. Int. Med., *102*:25, 1958.
21. Krall, L. P., White, P. and Bradley, R. F.: Diabetes, *7*:468, 1958.
22. Jelliffe, D. B. and Stuart, K. L.: Brit. Med. J., *1*:75, 1954.
23. Hassal, C. H., Reyle, K. and Feng, P.: Nature, *173*:356, 1954.
24. Holt, v. C. and Leppla, W.: Zeit. Physiol. Chem., *313*:277, 1958.
25. Chen, K. K., Anderson, R. C., McCowen, M. C. and Harris, P. N.: J. Pharmacol. Exp. Ther., *121*:272, 1957.
26. Feng, P. C. and Patrick, S. J.: Brit. J. Pharmacol. Chemother., *13*:125, 1958.
27. Janbon, M., Chaptal, J., Vedel, A. and Schaap, J.: Monpellier Med., *21*:441, 1942.
28. Loubatieres, A.: Presse Med., *63*:1701, 1955, and Ann. N. Y. Acad. Sci., *71*:4, 192, 1957.
29. McLamore, W. M., Fanelli, G. M., P'an, S. Y. and Laubach, G. D.: Ann. N. Y. Acad. Sci., *74*:443, 1959.
30. Garcia-Blanco, J. and Anton, V.: Rev. Espan. Fisiol., *14*:17, 119, 123, 1958.
31. Anton, V. and Gonzalez-Rey, M.: Bull. Soc. Chim. Biol., Suppl. *4*:45, 1957.
32. Meli, A., Parenti, M. A. and Capraro, V.: Il Farmaco, *12*:268, 1957.
33. Lee, C. C., Anderson, R. C. and Chen, K. K.: Arch. Intern. Pharmacodyn. Ther., *113*: 302, 1958.
34. Moss, D. G.: J. Clin. Path., *10*:371, 1957.
35. Foriet, A. A., Miller, W. L., Jr., Krake, J. and Struck, W. A.: Proc. Soc. Exp. Biol. Med., *96*:180, 1957.
36. Shepherd, H. G., Jr. and McDonald, H. J.: Clin. Chem., *4*:496, 1958.
37. McDonald, H. J. and Sawinski, V. J.: Texas Reports Biol. Med., *16*:479, 1958.
38. Lundbaek, K. and Nielsen, K.: Acta Endocrinol., *27*:325, 1958.
39. Stowers, J. M., Mahler, R. F. and Hunter, R. B.: The Lancet, *1*:278, 1958.
40. Stowers, J. M., Constable, L. W. and Hunter, R. B.: Ann. N. Y. Acad. Sci., *74*:689, 1959.
41. Forsham, P. H., Magid, G. J. and Dorosin, D. E.: Ann. N. Y. Acad. Sci., *74*:672, 1959.
42. Root, M. A.: J. Pharmacol. Exp. Therap., *119*:468, 1957.
43. Penhos, J. C.: Rev. Soc. Argentina Biol., *33*: 44, 1957.

44. West, K. M. and McCampbell, S. R.: Proc. Sci. Exp. Biol. Med., *98*:724, 1958.
45. Root, M. A., Sigal, M. V., Jr. and Anderson, R. C.: Diabetes, *8*:7, 1959.
46. Kirtley, W. R.: Diabetes, *6*:72, 1957.
47. Marble, A. and Camerini-Davalos, R.: Ann. N. Y. Acad. Med., *71*:239, 1957.
48. Mancini, R. E., Penhos, J. C., Gerschenfeld, H. M. and Isquierdo, I.: Rev. Soc. Argentina Biol., *33*:219, 1957.
49. Tuchmann-Duplessis, H. and Mercier-Parot, L.: C. r. Soc. Biol., *152*:460, 1958.
50. O'Donovan, C. J.: Diabetes (in press).
51. Skinner, N. S., Jr., Bolding, O. T., Hayes, R. L. and Hill, S. R., Jr.: Clin. Res., *7*:144, 1959.
52. Gerstenberg, E., Hasselblatt, A. and Schmidt, G.: Arch. Expr. Path. Pharmakol., *231*:407, 1957.
53. Dobson, H. L., Guilak, H., Carter, R. E., Montgomery, H. and Greene, J. A.: Ann. N. Y. Acad. Sci., *74*:940, 1959.
54. Signorelli, S.: Minerva Medica, *49*:1399, 1958.
55. Gordon, M. F., Buse, J. F. and Lukens, F. D. W.: Diabetes, *6*:7, 1957.
56. Houssy, B. A., Penhos, J. C., Teodosio, N., Bowkett, J. and Apelbaum, J.: Ann. N. Y. Acad. Sci., *71*:12, 1957.
57. Houssay, B. A., Penhos, J. C. and Apelbaum, J.: Rev. Soc. Argentina Biol., *33*:211, 1957.
58. Houssay, B. A. and Penhos, J. C.: C. r. Soc. Biol., *152*:1390, 1958.
59. Creutzfeldt, W.: Diabetes, *6*:135, 1957.
60. Goldner, M. G., Volk, B. W., Weisenfeld, S. W. and Lazarus, S. S.: Diabetes, *6*:53, 1957.
61. Gepts, W.: Contribution a l'etude morphologique des ilots de Langerhans au cours du diabete. Acta Medica Belaica. Publishers, Bruxelles; see also Endokrinologie, *36*:185, 1958.
62. Foa, P. P., Galansino, G. and Pozza, G.: Recent Progress Hormone Res., *13*:473, 1957.
63. Hokfelt, B. and Hultquist, G.: Acta Physiol. Scand., *43*:8, 1958.
64. Pfeiffer, E. F.: Meet. Div. Med. Chem., Amer. Chem. Soc., San Francisco, 1958.
65. Antonini, F. M., Caracristi, R. and Vallecorsi, G.: Minerva Medica, *49*:1525, 1958.
66. Vaugham, M.: Diabetes, *6*:16, 1957.
67. Miller, M. and Craig, J. W.: Metabolism, *5*:868, 1958.
68. Berson, S. A., Yalow, R. S., Weisenfeld, S., Goldner, M. G. and Volk, B. W.: Diabetes, *6*:54, 1957.
69. Volk, B. W., Goldner, M. G., Weisenfeld, S. and Lazarus, S. S.: Ann. N. Y. Acad. Sci., *71*:141, 1957.
70. Weber, G. and Cantero, A.: Metabolism, *7*:333, 1956.
71. Kuether, C. A., Scott, E. G., Martinez, C., Lee, H. M. and Pettinga, C. W.: Diabetes, *6*:23, 1956.
72. Cox, R. W. and Williams, R. H.: Diabetes, *6*:273, 1957.
73. Cahill, G. F., Jr., Ashmore, J., Renold, A. E. and Hastings, A. B.: Amer. J. Med., *26*:264, *5*:868, 1956.
74. Coltori, M., Giusti, G. and Ascione, A.: Minerva Medica., *49*:1499, 1958.
75. Mohnike, G., Knitsch, K. W., Boser, H., Werner, G. and Werner, S.: Deut. Med. Wschr., *82*:1580, 1957.
76. Knitsch, K. W.: Zeit. Physiol. Chem., *309*:184, 1957.
77. Kelsey Frey, I. and Wright, P. H.: Brit. J. Pharmacol., *12*:350, 1957.
78. Wallenfels, v. K., Summ, H. D. and Creutzfeldt, W.: Deut. Med. Wschr., *82*:1581, 1957.
79. Berson, S. A. and Yalow, R. S.: Diabetes, *6*:274, 1957.
80. Jacobs, G., Reichard, G., Goodman, E. H., Jr., Friedmann, B. and Weinhouse, S.: Diabetes, *7*:358, 1958.
81. Searle, G. L., Mortimore, G. E., Buckley, R. and Reilly, W. A.: Res. Proc., *6*:92, 1958.
82. Ashmore, J., Cahill, G. F., Jr., Earle, A. S. and Zottu, S.: Diabetes, *7*:1, 1958.
83. Tarding, F. and Schambye, P.: Endokrinologie, *36*:222, 1958.
84. Renold, A. E., Winegrad, A. I., Froesch, E. R. and Thorn, G. W.: Metabolism, *5*:757, 1956.
85. Corvilain, J., Tagnon, R. and Scoby, L.: Rev. Franc. Etudes Clin. Biol., *3*:613, 1958.
86. Leynse, B. and Ybema, H. J.: Clinica Chimica Acta, *2*:284, 1957.
87. Creutzfeldt, W. and Sutterle, H.: Deut. Med. Wschr., *82*:1574, 1957, and Ann. Endocrinol., *18*:184, 1957.
88. Perrini, M. and Rizzi, D.: Minerva Medica, *49*:1475, 1958.
89. Cascio, G.: Boll. Soc. It. Biol. Sper., *33*:1007, 1957.
90. Bressler, R. and Engel, F. L.: Proc. Soc. Exp. Biol. Med., *95*:738, 1957.
91. Henry, W. L., Kim, J. H. and Hall, A. S.: Amer. J. Physiol., *192*:514, 1958.
92. Ashmore, J., Cahill, G. F., Jr. and Earle, A. S.: Ann. N. Y. Acad. Sci., *71*:131, 1957.
93. Miller, W. L., Jr., Krake, J. J., Vander Brook, M. J. and Reineke, L. M.: Ann. N. Y. Acad. Sci., *71*:118, 1957, and J. Pharmacol. Exp. Ther., *119*:513, 1957.
94. Sobel, G. W., Rodriguez-Inigo, J., Morton, J. V. and Levine, R.: Metabolism, *7*:222, 1958.
95. Dulin, W. E. and Johnston, R. L.: Ann. N. Y. Acad. Sci., *71*:177, 1957.
96. Fritz, I. B., Weinstein, M., Morton, J. V. and Levine, R.: Endocrinology, *60*:76, 1957.
97. Schneider, J. A., Salgado, E. D., Jaeger, D. A. and Delahunt, C.: Ann. N. Y. Acad. Sci., *74*:427, 1959.
98. Mirsky, I. A., Perisutti, G. and Jinks, R.: Proc. Soc. Exp. Biol. Med., *91*:475, 1956.
99. Mirsky, I. A., Perisutti, G. and Gitelson, S.: Ann. N. Y. Acad. Sci., *71*:103, 1957.
100. Gonzalez-Rey, M. and Anton, V.: Rev. Espan. Fisiol., *13*:9, 195, 1957.
101. Yoshida, H., Yokoo, S., Aochi, O. and Uehira, T.: Nippon Naibunpi Gakki Zasshi, *34*:1, 1958.
102. Yoshida, H., Ishii, M., Migihashi, T., Takeshita, Y. and Masaki, K.: Nippon Naibunpi Gakkai Zasshi, *34*:705, 1958.
103. Strassle, R. and Pletscher, A.: Klin. Wschr., *14*:719, 1957.
104. Prout, T. E. and Evans, I. E.: Ann. N. Y. Acad. Sci., *74*:570, 1959.

105. Field, J. B. and Woodson, M. L.: Proc. Soc. Exp. Biol. Med., *93*:534, 1956.
106. Aiman, R. and Kulkarni, R. D.: Brit. J. Pharmacol. Chemother., *12*:475, 1957.
107. Houssay, B. A., Penhos, J. C., Urgoiti, E., Teodosio, N., Apelbaum, J. and Bowkett, J.: Ann. N. Y. Acad. Sci., *71*:25, 1957.
108. Caren, R. and Corbo, L.: J. Clin. Invest., *36*: 1546, 1957.
109. Urgoiti, E. J.: C. r. Soc. Biol., *152*:192, 1958.
110. Ricketts, H. T., Wildberger, H. L. and Schmid, H.: Ann. N. Y. Acad. Sci., *71*:170, 1957.
111. Volk, B. W. and Lazarus, S. S.: Diabetes, 7:125, 1958.
112. Kracht, J., Kroner, B., Holt, v. L. and Holt, v. C.: Naturwiss, *44*:16, 1957.
113. Lazarus, S. S. and Volk, B. W.: Endocrinology, *62*:292, 1958.
114. Klimas, J. E., Jr. and Searle, G. W.: Feder. Proc., *17*:87, 1958.
115. Pozza, G., Galansino, G. and Foa, P. P.: Proc. Soc. Exp. Biol. Med., *93*:539, 1956.
116. Goetz, F. C. and Egdahl, R. H.: Feder. Proc., *17*:55, 1958.
117. Holt, v. C., Holt, v. L., Kracht, J. Kroner, B. and Kuhnau, J.: Science, *125*:735, 1957.
118. Candela, J. L. R. and Candela, R. R.: Rev. Iberica Endocrinol., *4*:413, 1957.
119. Weaver, J. A., Prout, T. E., Scott, G. W. and Asper, S. P.: Brit. Med. J., *1*:425, 1958.
120. Seltzer, H. S. and Smith, W. H.: J. Lab. Clin. Med., *52*:945, 1958.
121. Colwell, A. R., Jr., Colwell, J. A. and Colwell, A. R., Sr.: Ann. N. Y. Acad. Sci., *71*:125, 1957.
122. Cappelli, V., Dozio, G. and Noli, S.: Boll. Soc. It. Biol. Sper., *9*:916, 1956.
123. Foa, P. P., Galansino, G., D'Amico, G. and Kanameishi, D.: Ann. N. Y. Acad. Sci., *74*: 570, 1959.
124. Galansino, G., Kanameishi, D. and Foa, P. P.: Metabolism (in press).
125. Meli, A., Piccinini, F., Parenti, M. A. and Capraro, V.: Naturwiss., *45*:135, 1958.
126. Piccinini, F., Parenti, M. A., Meli, A. and Capraro, V.: Il Farmaco, *12*:602, 1957.
127. Unger, R. H. and Madison, L. L.: J. Clin. Invest., *37*:627, 1958.
128. Clarke, D. W. and Senman, H.: Diabetes, 7:283, 1958.
129. Candela, J. L. R. and Candela, R. R.: Rev. Iberica Endocrinol., *4*:309, 1957.
130. Lundbaek, K., Nielsen, K. and Rafaelsen, O. J.: Ann. N. Y. Acad. Sci., *74*:419, 1959.
131. Garattini, S., Paoletti, R. and Tessari, L.: Arzneimittel-Forsch., *8*:477, 1958.
132. Pletscher, A. and Gey, K. F.: Experientia, *13*:447, 1957.
133. Gourley, D. R. H. and Dodd, R. H.: Amer. J. Physiol., *192*:471, 1958.
134. Goetz, F. C., Gilbertsen, A. S. and Josephson, V.: Metabolism, *6*:788, 1956.
135. Madison, L. L. and Unger, R. H.: Metabolism, 7:227, 1958.
136. Butterfield, J., Frey, K. I. and Holling, E.: Diabetes, 7:449, 1958.
137. Purnell, R., Arai, Y., Pratt, E., Hlad, C., Jr. and Elrick, H.: Metabolism, *5*:744, 1956.
138. Craig, J. W., Molvahn, V. J., Woodward, H., Jr. and Miller, M.: Diabetes, 7:267, 1958.
139. Meli, A., Piccinini, F., Parenti, M. A. and Capraro, V.: Il Farmaco, *12*:274, 1957.
140. D'Amico, G., Galansino, G. and Foa, P. P.: Endokrinologie, *36*:219, 1958.
141. Galansino, G., D'Amico, G., Kanameishi, D. and Foa, P. P.: Proc. Soc. Biol. Exp. Biol. Med., *99*:447, 1958.
142. Gambassi, G. and Pirrelli, A.: Arch. Pat. Clin. Med., *34*:161, 1957.
143. DeMeutter, R. C., Khachadurian, A. K. and Marble, A.: Proc. Soc. Exp. Biol. Med., *99*: 33, 1958.
144. Bierman, E. L., Roberts, T. N. and Dole, V. P.: Proc. Soc. Exp. Biol. Med., *95*:437, 1957.
145. Segal, S., Frawley, T. F. and Foley, J.: Diabetes, *6*:422, 1957.
146. Vallecorsi, G., Caracristi, R. and Antonini, F. M.: Minerva Medica, *49*:1532, 1958.
147. Mohnike, G., Czyzyk, A. and Bibergeil, H.: Deut. Med. Wschr., *82*:1579, 1957.
148. Szucs, S. and Tiszai, A.: Diabetes, 7:288, 1958.
149. Izzo, J. L.: Diabetes, *6*:45, 1957.
150. Madison, L. L. and Unger, R. H.: J. Clin. Invest., *37*:631, 1958.
151. Kracht, J., Holt, v. C. and Holt, v. L.: Enkrinologie, *34*:129, 1957.
152. Creutzfeldt, W. and Geginat, G.: Arzneimittel-Forsch., *8*:464, 1958.
153. Gonnard, P., Dalion, J. and Thevenoux, A. M.: Presse Med., *65*:777, 1957.
154. Pfeiffer, E. F., Steigerwald, H., Sandritter, W., Bander, A., Maaer, A., Becker, U. and Retiene, K.: Deut. Med. Wschr., *82*:1568, 1957.
155. Scholer, H. F. L. and Gaarenstroom, J. H.: Acta Endocrinol., *29*:147, 1958.
156. Kramer, D. W. and Perilstein, P. K.: Diabetes, 7:384, 1958.
157. Introzzi, P., Bernasconi, C. and Buscarini, L.: Acta Med. Scand., *160*:59, 1958.
158. Zeffren, J. L. and Sherry, S.: Metabolism, *6*:504, 1957.
159. Fabrykant, M.: Metabolism, *6*:509, 1957, and 7:213, 1958.
160. Beaser, S. B.: New England J. Med., *259*: 1207, 1958.
161. Volk, B. W. and Lazarus, S. S.: Amer. J. Med. Sci., *237*:1, 1959.
162. Rayreuther, H.: Arch. Psych. Nervenkrankh., *195*:435, 1957.
163. Ungar, G.: Personal communication.
164. Madison, L. L. and Unger, R. H.: Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)," Houston, 1959.
165. Krall, L. P. and Bradley, R. F.: Ann. Int. Med., *50*:586, 1959.
166. Coates, J. R. and Robbins, J. J.: J.A.M.A., *170*:941, 1959.

EXPERIMENTAL DIABETES: A HISTORY OF BASIC RESEARCH

STEPHEN R. GREENBERG, Ph.D.*

The great advances, the rapid progress and the far-reaching achievements in all areas of scientific endeavor cannot help but leave their impress upon the individual and upon the society in which he finds himself. Glowing, stirring accounts of these attainments not infrequently distort his perspective of the firm foundations on which they rest. These foundations have been laid and raised through the efforts and labors of countless other individuals who have contributed in minute ways toward the construction of the scientific edifice. Such contributions are in the form of ideas — concepts derived through research — which in due course are combined with others to form a vast pool to be tapped as man's scientific frontiers expand.

In no other field of endeavor as in medicine has basic research been of such paramount importance. All of the forward steps taken in the alleviation of human suffering have had their roots in the small sums of knowledge added and re-added by dedicated individuals.

We have at hand no finer example than that of the history of Diabetes Mellitus. In the study of any disease process, it is well to begin with the patient who has been afflicted — a *clinical* study. The close observation of the signs and symptoms, the examination of the lesions which produce them and the effects of treatment should all be considered. Such activities, however, provide only a segment of knowledge. One must search further; he must follow the course of the disease as it moves from its inception. Because this cannot be accomplished in man, attempts must be made to reproduce the disease in the laboratory animal where its time of origin can be ascertained and its course and progress charted and scrutinized. It is here that experimental diabetes takes root. A history of the laboratory study of this disease is truly a history of all basic research.

Pancreatectomy

It was in the spring of 1889 that Von Mehring and Minkowski attempted, while working on experiments with lipid enzymes to remove the pancreas from a dog. They had been warned previously, by no less a personage than Claude Bernard, that no animal could possibly survive such an ordeal. However, this dog did survive and, to the surprise, even consternation of Minkowski (Von Mehring appeared to have soon lost his interest in the enterprise)[1], it developed glycosuria.

Early it became apparent that if similar animals were to live and become useful research tools, less radical means must be employed. Subtotal pancreatectomy came into prominence when it was proven that by this procedure diabetes could be successfully established in viable animals[2]. The way was now open for the classical morphologic descriptions of the islet lesions in the dog by Allen[3] and in the dog and cat by Homans[4,5]. The loss of granules from the beta cell, the swelling and vacuolation of its cytoplasm and the eventual disintegration of the cell were well documented.

Thus it now became possible to observe the early effect of diabetes on the pancreas. These experiments were, notwithstanding, drastic procedures, and the conclusions so obtained could not be accurately predicated on the human subject where the organ, though diseased, remains within the body.

Glucose Administration

Woerner[6], in 1938, gave continuous intravenous injections of dextrose to a series of guinea pigs. Though marked hyperglycemia supervened, the pancreata of these animals revealed the degranulation and disappearance of their beta cells. Astonishingly, the islets seemed to increase in size and new beta cells arose through mitoses of pre-existing

* Associate in Pathology, The Chicago Medical School, Chicago, Ill.

ones. Within a month, the islets were almost restored and the guinea pigs gave no evidence of diabetes. These observations were confirmed the following year by Gomori[7]. It was now manifest that diabetes could be reproduced in an animal as a consequence of glucose administration.

When Dohan and Lukens reported the results of their investigations[8], the oftennoted beta cell degranulation took on added significance. It appeared that these cells were under a functional strain due to the increased load of carbohydrate placed into the circulation, and many of them became exhausted; the loss of granules was correlated with a decrease in available insulin[9,10]. This issue, however, was obscured by later evidence[2].

Such basic observations as these introduced a broader perspective. Another link was added to this complex chain of deranged animal metabolism.

Alloxan

A real impetus in the basic research of diabetes, and indeed of all metabolic disorders, was provided in 1943 when Dunn and his associates[11] announced that the injection of alloxan, a derivative of normal purine metabolism, into rabbits brought about severe hyperglycemia and destruction of the pancreatic islets. Other investigators soon verified these findings[12,13,14].

Employing this substance, Goldner and Gomori[15] established diabetes in the dog. The animals were preserved for considerable periods and, when sacrificed, the salient histological feature was found to be the disappearance of the beta cells. They also noted a marked vacuolation of the pancreatic duct epithelium, an observation of considerable significance to later investigators.

Because it had been demonstrated that the intrinsic pancreatic action of alloxan was both rapid and specific, the issue was now concerned with the earliest islet cell changes. Beginning degranulation of the beta cells occurs within five minutes[16]. The cytoplasm begins to fragment within eight hours and nuclear pyknosis soon follows; the cell then shrinks, and by the fourth or fifth day after alloxanization, only occasional beta cells are discernable[14]. Hydropic degeneration of the islet cells was described by Kennedy and Lukens[17], but was reported by them to be transitory in nature. The alpha cells remain essentially unaffected[18,19,20].

Other organs are also influenced by this substance. The renal tubular eithelium shows focal or extensive hydropic degeneration and glycogen infiltration[21,22,23]. The liver is injured also. There are foci of necrosis scattered throughout the parenchyma and glycogen is lost from the hepatic cells.

In 1947, Duff and his co-workers[24] described extensive hydropic degeneration in the pancreatic islets and ductules of chronically diabetic rabbits that persisted despite the long duration of the disease, but yet was reversible with insulin treatment. Similar observations were made in the pancreas of the cat by Haist[25]. The exact nature of this co-called "hydropic degeneration" of the pancreatic islets was elucidated in the course of the interesting studies of Toreson[26] who, by appropriate histologic technics, established beyond all question that the vacuoles in the alloxanized pancreas were not "hydropic," but were instead the result of artifactual removal of intracytoplasmic masses or accumulations of glycogen rather than the accumulation of excessive quantities of serous fluid. He introduced the term, "Glycogen infiltration." On the basis of this investigation, it appears quite likely that in Diabetes Mellitus, there is a progressive infiltration of glycogen into the pancreas, as observed also in the human organ by Toreson.

Recognizing the significance of this study, Lazarus and Volk[27] imply that the concept of hydropic degeneration representing functional exhaustion of the beta cell is no longer tenable, because vacuolation of this cell, rather than representing a degenerating lesion due to such exhaustion, is in reality an expression of glycogen infiltration as a consequence of hyperglycemia. It is proposed instead that degranulation and vacuolation of beta cells not be considered as sequential events, but instead as independent manifestations of hyperglycemia.

Johnson[28] noted that in the alloxanized guinea pig restoration of the injured islet tissue occurred within four days. This, he speculated, was attributable to: 1) transformation of peri-insular acinar cells into islet cells by what he termed, "redifferentiation," 2) transformation of acini into new islets and 3) proliferation of the epithelium of the smallest ducts to form new islets. Islet degeneration by the proliferation of pre-existing beta cells, as previously described, was now thought to play but a minor role. More recent evidence indicates that at least part of the beta cells in regenerating islets arise from ductule epithelium of the pancreas[29].

With the continuing improvement of technics and equipment, such concepts as these will be expanded and amplified in the light of ultramicroscopic studies[30].

At this stage, the picture is at once becoming clear and also complex. More building blocks are now to be added to the temple of diabetic understanding.

Pituitary Diabetes

The relationships between the anterior pituitary gland and the pancreas have long been investigated. In 1927, Johns and his associates[31] produced hyperglycemia, polyuria and glycosuria in dogs following the injection of extracts of the anterior lobe of the ox pituitary gland. The further relation of this portion of the gland to carbohydrate metabolism was suspected on the basis of the often-noted occurrence of hyperglycemia and glycosuria in acromegaly. The observations of Johns and his group were subsequently confirmed in the dog and in the rabbit[32,33].

An important link in this chain was supplied by Houssay and Biasotti[34] when it was shown by them that diabetes could be prevented in toads by the removal of the anterior lobe of the pituitary; furthermore, subcutaneous implantation of the gland in these animals re-established the diabetes.

That pituitary diabetes could be made permanent with destruction of the pancreatic islets was proven by Young[35] who employed daily injections of anterior lobe extract.

Attention became critically focused on the role of the anterior pituitary gland in diabetes by the monumental researches of Houssay[36]. This investigator reported that the removal of the pancreas of an animal from which the pituitary gland had previously been extracted resulted in a more attenuated diabetes than that which followed pancreatectomy in a normal dog.

Interest now centered on a more precise study of the hormones produced by the anterior lobe of the pituitary. The question arose as to which one(s) of the secretions elaborated by this organ are actually concerned with diabetes. Bennet and Li[37] induced increased glycosuria in diabetic rats by the injection of purified adrenocorticotropic hormones. A similar diabetogenic effect was recorded in normal and in partially depancreatized animals by other investigators[38,39]. A temporary diabetic state has recently been produced in normal human subjects by the injection of ACTH[40].

Purified growth hormone has likewise been shown to have potent diabetogenic properties[41] in intact and in partially depancreatized rats[42].

The thyrotropic, lactogenic and gonadotropic factors of the anterior lobe do not possess any diabetogenic properties.

Thyroid Diabetes

It is known that the feeding of thyroid tissue to normal animals may be subsequently followed by transitory hyperglycemia. Permanent diabetes cannot be induced, however. If the pancreas is in some way injured, either by partial removal, the administration of alloxan or by the injection of anterior pituitary extracts, thyroid feeding will produce hyperglycemia and, if continued sufficiently, a lasting diabetes with glycosuria, ketonuria and characteristic beta cell alterations. Experimental diabetes produced in this fashion will persist even when the administration of thyroid has ceased. This appears to indicate that the thyroid role is supplementary. Such persistance is termed by Houssay[43] "metathyroid diabetes."

A diet rich in carbohydrates favors the onset of diabetes, and the administration of insulin will prevent its occurrence

but will not effect a cure once the disease has become established. It has been demonstrated that the removal of the thyroid gland simultaneously with partial pancreaectomy in an experimental rat will circumvent the appearance of this disease in the animal.

The true bonds between thyroid and pancreas and the mechanisms which result in diabetes are still obscure. Long continued hyperglycemia may, as has been suggested, exert some injurious effect upon the beta cells[2], but much has yet to be uncovered in this search.

Steroid Diabetes

That a connection exists between the adrenal gland and diabetes was clearly established by the important work of Long[44] who demonstrated that coincident with the removal of the adrenal glands from a cat, in which diabetes had previously been produced by pancreatectomy, there was a striking decrease in the excretion of glucose and acetone. The significant role played by the adrenal cortex was further shown when the denervation or demedullation of the glands failed to alleviate the condition. These observations were strengthened when evidence was introduced that the administration of corticosteroids to intact and to partially depancreatized rats would produce or increase glycosuria[45,46].

When purified adrenal cortical hormones became available, impetus was given to the production of diabetes with these substances. Corticosterone, 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone have all been found to possess diabetogenic action[43]. The mechanisms involved are under current study. The corticosteroids appear to exert at least some effect by stimulating gluconeogenesis from protein and perhaps also by inhibiting the proper utilization of body carbohydrate.

The pancreatic islets of cortisone-treated animals exhibit striking alterations. These are: 1) degranulation and glycogen infiltration of the beta cells;)2 glycogen infiltration of the cells in the intercalated ducts; 3) diffuse proliferation of the small ductules of the pancreas and the intra-islet vessels and 4) changes in size (hypertrophy) and shape of the islets.

Though both ACTH and cortisone have been proven to produce similar effects upon the islet cells[51], the influence of the adrenal glands upon the pancreas cannot be distinctly separated from those possibly exerted by the adrenocortropic hormone of the anterior lobe of the pituitary. The roles of both are indeed complex.

Other Diabetogenic Agents

Uric Acid

There is experimental evidence that uric acid, which is chemically related to alloxan, will produce in animals hyperglycemia and glycosuria if the blood glutathione level has been previously lowered by suitable dietary measures. Islet cell lesions, histologically similar to those in mild alloxan diabetes, have been described[52,53].

Estrogens

Estrogenic hormones, especially stilbesterol, are able to stimulate hyperglycemia and glycosuria in partially depancreatized rats. Massive doses of testosterone have a similar, though less pronounced diabetogenic action[45].

Though the diabetogenic action of the hormones of the anterior pituitary gland and the adrenal cortex may be perceived because of the close relationship they bear to carbohydrate metabolism, the observed effects of estrogens are far less comprehendable. Ingle[54], noting a similar hyperglycemic consequence with estrogenic hormones and with adrenal steroids, postulated that since the cortices of the adrenals enlarge during the administration of estrogens, the observed diabetogenic effect of these substances may be the result of an increased formation of steroids. Experimental evidence, however, did not lend support to such an idea.

Partially depancreatized rats were made diabetic by the daily injection of diethylstilbesterol; glycosuria stopped when these injections were discontinued. The animals were then adrenalectomized; glycosuria again followed hormone injection and was checked by its withdrawal. When these animals were given main-

tenance doses of cortical steroids or sodium chloride, the diabetogenic effects of the estrogenic hormone was slight or absent. It was thus established that the presence of the adrenal cortical hormones is essential for a full manifestation of the diabetogenic action of estrogens, but a change in the secretory activity of the adrenal cortex did not appear essential for its manifestation.

Recent evidence indicates that estradiol, alone and in combination with insulin, may induce a moderation of experimentally produced (alloxan) diabetes and histologic recovery of the beta cells[55]. Houssay[56] has demonstrated that estrogens decreased the incidence of diabetes in subtotally pancreatectomized rats while androgens (testosterone) brought about an increase in the incidence and severity of the condition. Other steroids (progesterone and desoxycorticosterone) exerted no effect. The protective action of estrogens is attributed to the fact that they induce hypertrophy and hyperplasia of the pancreatic islets.

Organic Reagents

Using as a basis the fact that zinc has been detected histochemically in the pancreatic islets, Kodota[57] has produced hyperglycemia and beta cell necrosis by the intravenous injection of 8-hydroxyquinoline and diphenylthiocarbazone, chemical agents which are able to bind zinc rendering it unavailable for metabolic purposes.

Ascorbic Acid Derivatives

Ascorbic acid is present in the body in an oxidized form known as dehydroascorbic acid. This substance possesses many chemical similarities to alloxan. Patterson[58], has been able to induce permanent hyperglycemia by the injection of dehydroascorbic acid. He also reported that greatly reduced amounts of alloxan are required to produce diabetes in rats if simultaneous injections of dehydroascorbic acid are given to the animals. Thus, this latter substance would appear to possess synergistic action with alloxan.

Spanning the Gulf

In this brief summary, an endeavor has been made to trace the development of basic research in Diabetes Mellitus and to further show how the contributions which have been forthcoming are singularly and mutually effective in advancing the understanding of this disease. At this juncture, it would seem desirable to comment on the impact of such laboratory study upon the human illness.

The question, for instance, arises as to whether the earliest experimental changes in the diabetic organ have their counterpart in the human pancreas. It is known that many of the latter tissue alterations are morphologically identical. Soon after its introduction as a diabetogenic agent, Brunschweig[59] attempted the use of alloxan in the treatment of a human islet cell tumor. This attempt did not meet with success, and it was concluded that the human species, at least, was immune to the effects of this substance. Later studies by Conn and his associates[60] demonstrated that necrosis could be induced in normal intact beta cells, though it required large quantities of the drug to do so.

Further, it has been found possible to induce diabetes in normal human subjects by the administration of adrenocorticotropic hormone, the condition lasting as long as this material is injected. Because the severity of the disease increased with the duration of this treatment, it appears possible to cause in man a permanent state of diabetes. Conn and co-workers have observed, in addition to derangements in glucose tolerance and glycosuria, an increased excetion of uric acid. It has been postulated that ACTH is responsible for larger concentrations of the intermediaries of purine metabolism, especially uric acid, and a fall in the level of blood glutathione. The beta cells are particularly sensitive to such changes and the production or release of insulin may consequently be affected[61,62].

Thus the relation of basic research to the clinical aspect of diabetes becomes more significant with the passage of time. In spanning the chasm between laboratory and clinic, sight must not be lost of the fact that though the disease processes may appear similar, the host organisms are not. As Goldner[63] has

recently stated, the human organism is subject to stress of many sorts, all of which leave their mark upon disease and modify it in ways not always reproducible in the laboratory.

Lastly, in any discourse on diabetes, one must never forget the truly monumental bridge erected between laboratory and clinic by Banting and Best who, in 1921, discovered insulin.

This then is the real significance of experimental diabetes, indeed of all basic research; for it is not how far we have come but in what direction we are moving, that is the final, ultimate measure of our progress.

BIBLIOGRAPHY

1. Houssay, B. A., The Discovery of Pancreatic Diabetes, Diabetes *1*:112-116, 1952
2. Warren, S., and Le Compte, P. M., The Pathology of Diabetes Mellitus, Third Edition, Lea & Febiger, Philadelphia, 1952.
3. Allen, F. M., Studies Covering Glycosuria and Diabetes, Cambridge, 1913.
4. Homans, J., Degeneration of the Islands of Langerhans Associated with Experimental Diabetes in the Cat, J. Med. Res., *30*:49-68, 1914.
5. A study of Experimental Diabetes in the Canine and Its Relation to Human Diabetes, J. Med. Res., *33*:1-51, 1915.
6. Woerner, C. A., Studies of the Islands of Langerhans After Continuous Intravenous Injection of Dextrose, Anat. Rec., *71*:33-57, 1938.
7. Gomori, G., Friedman, N. B., and Caldwell, P. R., Beta Cell Changes in Guinea Pig Pancreas in Relation to Blood Sugar, Proc. Soc. Exp. Biol. and Med., *41*:567-570, 1939.
8. Dohan, F. C., and Lukens, F. D. W., Lesions of the Pancreatic Islets Produced in Cats by Administration of Glucose, Science, *105*: 183, 1947.
9. Barron, S. S., and State, D., Effect of Prolonged Intravenous Administration of Dextrose on Beta Cells of Islets of Langerhans, Arch. Path., *48*:299-304, 1949.
10. Brown, E. M., Dohan, F. C., Freedman, L. B., De Moor, F., and Lukens, F. D. W., The Effects of Infusion of the Dog's Pancreas with Glucose, Endocrinol., *50*:644-656, 1952.
11. Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., Necrosis of Islets of Langerhans Produced Experimentally, Lancet, *I*:484-487, 1943.
12. Brunschweig, A., Allen, J. G., Goldner, M. G., and Gomori, G., Alloxan, JAMA, *122*: 966, 1943.
13. Bailey, C. C., and Bailey, O. T., The Production of Diabetes Mellitus in Rabbits with Alloxan, JAMA, *122*:1165-1166, 1943.
14. Gomori, G., Production of Diabetes Mellitus in Rats with Alloxan, Proc. Soc. Exp. Biol. and Med., *54*:287-290, 1943.
15. Goldner, M. G., and Gomori, G., Alloxan Diabetes in the Dog, Endocrinol., *33*:297-308, 1943.
16. Hughes, H., and Ware, L. L., Diabetogenic Action of Alloxan, Lancet, *I*:148-150, 1944.
17. Kennedy, W. B., and Lukens, F. D. W., Observations on Alloxan Diabetes, Proc. Soc. Exp. Biol. and Med., *57*:143-149, 1944.
18. Dunn, J. G., Duffy, E., Gilmour, M. K., Kirkpatrick, J., and McLetchie, N. G. B., Further Observations on the Effects of Alloxan on the Pancreatic Islets, J. Physiol., *103*:233-243, 1944.
19. Hard, W. L., and Carr, C. J., Experimental Diabetes Produced by Alloxan, Proc. Soc. Exp. Biol. and Med., *56*:214-216, 1944.
20. Duffy, E., Alloxan Diabetes in the Rabbit, J. Path. and Bact., *57*:199-212, 1945.
21. Dunn, J. S. Kirkpatrick, J., McLetchie, N. G. B., and Telfer, S. V., Necrosis of the Islets of Langerhans Produced Experimentally, J. Path. and Bact., *55*:245-257, 1943.
22. Duff, G. L., and Starr, H., Experimental Alloxan Diabetes in Hooded Rats, Proc. Soc. Exp. Biol. and Med., *57*:280-282, 1944.
23. Lazarow, A., and Palay, S. L., The Production and Course of Alloxan Diabetes in the Rat, J. Lab. and Clin. Med., *31*:1004-1015, 1946.
24. Duff, G. L., McMillan, G. C., and Wilson, D. C., Hydropic Changes in Pancreatic Ductules and Islets in Alloxan Diabetes in the Rabbit, Proc. Soc. Exp. Biol. and Med., *64*:251-255, 1947.
25. Haist, R. E., Studies in Experimental Diabetes, Am. J. Med., *7*:585-595, 1949.
26. Toreson, W. E., Glycogen Infiltration (So-Called Hydropic Degeneration) in the Pancreas in Humans and Experimental Diabetes Mellitus, Am. J. Path., *27*:327-348, 1951.
27. Lazarus, S. S., and Volk, B. W., Glycogen Infiltration (Hydropic Degeneration) in the Pancreas, Arch. Path., *66*:59-71, 1958.
28. Johnson, D. D., Alloxan Administration in the Guinea Pig, Endocrinol., *47*:393-398, 1950.
29. Bencosme, S. A., Cytology of Islet Cells in Alloxan Diabetic Rabbits, Am. J. Path., *31*: 1149-1163, 1955.
30. ——, and Pease, D. C., Electron Microscopy of the Pancreatic Islets, Endocrinol., *63*:1-13, 1958.
31. Johns, W. S., O'Mulvenny, T. O., Potts, E. B., and Laughton, N. B., Studies on the Anterior Lobe of the Pituitary Body, Am. J. Physiol., *80*:100-106, 1927.
32. Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., Disturbance of Carbohydrate Metabolism in Normal Dogs Injected with Hypophyseal Growth Hormone, Proc. Soc. Exp. Biol. and Med., *29*:857, 1932.

33. Baumann, E. J., and Marine, D., Glycosuria in Rabbits Following Injections of Saline Extract of Anterior Pituitary, Proc. Soc. Exp. Biol. and Med., *29*:1220-1223, 1932.
34. Houssay, B. A., and Biasotti, A., Hypophysectomie et Diabete Pancreatique Chez de Crapaud, Compt. Rend. Soc. de Biol., *104*: 407-410, 1930.
35. Young, F. G., Permanent Experimental Diabetes Produced by Pituitary (Anterior Lobe) Injections, Lancet, *II*:372-374, 1937.
36. Houssay, B. A., Carbohydrate Metabolism, New Eng. J. Med., *214*:971-983, 1936.
37. Bennet, L. L., and Li, C. H., The Effects of the Pituitary Growth and Adrenocorticotropic Hormones on the Urinary Glucose and Nitrogen of Diabetic Rats, Am. J. Physiol., *150*:400-404, 1947.
38. Campbell, J., Davidson, I. W. F., Snair, W. B., and Lei, H. P., Diabetogenic Effects of Purified Growth Hormone, Endocrinol., *46*: 273-281, 1950.
39. Houssay, B. A., and Anderson, E., Diabetogenic Action of Purified Anterior Pituitary Hormones, Endocrinol., *45*:627, 1949.
40. Conn, J. W., Lewis, L. H., and Johnson, W. W., Alleviation of Experimental Diabetes in Man by Administration of Reduced Glutathione (GSH): Metabolic Implications, Science, *109*:279, 1949.
41. Cotes, P. M., Reid, E., and Young, F. G., Diabetogenic Action of Pure Anterior Pituitary Growth Hormone, Nature, *164*:209-211, 1949.
42. Bennet, L. L., and Evans, H. M., The Hypophysis and Diabetes Mellitus, Chapter VII, Vol. II, The Hormones, ed. by G. Pincus, and K. V. Thimann, Academic Press, New York, 1950.
43. Houssay, B. A., Recent Progress in Hormone Research, Vol. II, ed. by G. Pincus, Academic Press, New York, 1948.
44. Long, C. H. N., and Lukens, F. D. W., The Effects of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat, J. Exp. Med., *63*:465-490, 1936.
45. Ingle, D. J., Diabetogenic Effect of Stilbesterol in Force-Fed Normal and Partially Depancreatized Rats, Endocrinol., *29*:838-848, 1941.
46. Long, C. H. N., Katzin, B., and Fry, E. G., The Orenal Cortex and Carbohydrate Metabolism, Endocrinol., *26*:309-344, 1940.
47. Kobernick, S. D., and More, R. H., Diabetic State with Lipaemia and Hydropic Changes in the Pancreas Produced in Rabbits by Cortisone, Proc. Soc. Exp. Biol. and Med., *74*:602-605, 1950.
48. Lazarus, S., and Bencosme, S. A., Alterations of Pancreas During Cortisone Diabetes in Rabbits, Proc. Soc. Exp. Biol. and Med., *89*:114-118, 1955.
49. Hausberger, F. X., and Ramsay, A. J., Effects of Cortisone Administration on Blood and Urinary Glucose, Nitrogen Excretion, Fat Deposition, and the Islets of Langerhans, Endocrind., *53*:423-435, 1953.
50. Bencosme, S. A., and Lazarus, S. S., The Pancreas in Cortisone-Treated Rabbits, Arch. Path., *62*:285-295, 1956.
51. Kinash, B., and Haist, R. E., Effect of ACTH and Cortisone on the Islets of Langerhans and the Pancreas in Intact and Hypophysectomized Rats, Am. J. Physiol., *178*:441-444, 1954.
52. Griffiths, M., Uric Acid Diabetes, J. Biol., Chem., *172*:853, 1948.
53. The Mechanism of the Diabetogenic Action of Uric Acid, J. Biol. Chem., *184*:289-298, 1950.
54. Ingle, D. J., Recent Progress in Hormone Research, Vol. II, ed. by G. Pincus, Academic Press, New York, 1948.
55. Rodriguez, R. R., Alloxan Diabetes in the Rat: Recovery Following Estrogen Treatment, Endocrinol., *55*:1-9, 1954.
56. Houssay, B. A., Action of Sex Hormones on Experimental Diabetes, Brit. Med., J., 2:505-510, 1951.
57. Kodota, I., Studies on Experimental Diabetes Mellitus as Produced by Organic Reagents, J. Lab. nad Clin. Med., *35*:568-591, 1950.
58. Patterson, J. W., The Diabetogenic Effect of Dehydroascorbic and Dehydroisoascorbic Acids, J. Biol. Chem., *183*:81-88, 1950.
59. Brunschweig, A., Allen, J. G., Owen, F. M., and Thornton, T. F., Alloxan in the Treatmen tof Insulin Producing Islet Cell Carcinoma of Pancreas, JAMA, *124*:212-216, 1944.
60. Conn, J. W., Hinerman, D. L., and Buxton, R. W., Effects of Alloxan upon the Human Pancreas, J. Lab. and Clin. Med., *32*:347, 1947.
61. ——, Louis, L. H., Johnson, M. W., Studies upon Mechanisms Involved in the Induction with Adrenocorticotropic Hormone of Temporary Diabetes Mellitus in Man, Proc. Am. Diab. Assoc., *8*:213-239, 1948.
62. ——, ——, and Wheeler, C. E., Production of Temporary Diabetes Mellitus in Man with Pituitary Adrenocorticotropic Hormone: Relation to Uric Acid Metabolism, J. Lab. and Clin. Med., *33*:651-661, 1948.
63. Goldner, M. G., Stress, Corticoids and Diabetes, Diabetes, *7*:410-413, 1958.

HORMONAL INTERRELATIONS *

HUGO R. RONY, M.D.**

Hormones may affect endocrine glands and actions of other hormones in various ways. There is a well-known pattern of interactions, the so-called "feedback mechanism" through which a hormone can inhibit its release from the gland that produces it; this appears to serve a purpose in that it is the basis of an automatic regulation of hormone production. Other hormonal interactions show no easily recognizable patterns or purposes and have to be learned one by one.

A hormone may stimulate or inhibit other glands than its own. It may act directly on another gland, or indirectly by way of a third gland's hormone. It may support or antagonize actions of other circulating hormones. Most hormones normally act on more than one target organ, and this gives rise to further variations in hormonal relations. Thus, the same two hormones may act synergistically on certain targets, and antagonistically on other targets. An interaction between two hormones on the same target may be different in different animal species; and even in the same species there may be a difference, depending on the concentration of the hormone in the blood (e.g., low levels of the hormone stimulating another gland, high levels of it inhibiting the same gland) or on the age of the subject (the interaction being different in the young from that in the adult). Finally, some hormones may be converted in the body into other hormones.

A comprehensive review of such vast and complex field cannot be compiled within the space available for this paper, but an attempt will be made to present briefly the most important hormonal interactions with emphasis on those that are significant for the diagnosis and therapy of endocrine disturbances in man.

The following system of presentation is adopted: Each hormone is dealt with in a separate chapter listing all its interactions with other glands and hormones; if the interaction is reciprocal, the reverse effect is again listed in the chapter on the other hormone. To facilitate convenient and quick orientation, a summary of interactions appears at the end of most chapters; however, many interactions are conditional and should be qualified or interpreted by referring to the text of the chapter. In terminology, "release" of the hormone from the gland, rather than "production" of the hormone in the gland, will be used throughout the text, since direct assays of hormone production are rarely available — especially in man — and release usually includes, or is proportional with, production.

I. THE GROWTH HORMONE (GH)

Relatively little is known about the interactions of GH with other glands and hormones for the following reasons: Purified preparations of this hormone have only recently become available; quantitative assays in the blood and urine have not been developed and as a result such basic questions as to whether the hormone is secreted intermittently or continuously and whether it is essential to all phases of growth or only to some, remain unanswered; there is a considerable degree of specificity among GH preparations made from pituitaries of different species.

Recently, GH was prepared from human pituitary glands and it was found that when administered to human pa tients the effects were not identical with those produced by beef or sheep GH (which were used in all earlier work); even human and monkey GH acted somewhat differently[1].

There is no evidence that GH has any effect on the production of action of anterior or posterior pituitary hormones.

1. GH and Thyroxine

GH is not known to influence the production of thryroxine but the two hormones may act synergistically on grow-

* From the Department of Medicine, The Chicago Medical School, and Mount Sinai Hospital.
** Clinical Associate Professor of Medicine, The Chicago Medical School; Senior Attending Physician in Endocrinology and Metabolism, Mount Sinai Hospital.

ing tissues; GH is more important for general growth, and thyroxine is more important for maturation. Growth occurs in the absence of the thyroid gland, but optimal growth requires presence of thyroxine as shown by the accelerated growth of the athyrotic or cretin child when treated with thyroxine; and the hyperthyroid child often shows excessive growth. When growth is orderly, thyroxine and GH produce orderly proliferation of cells as a result of stimulation of protein anabolism; in the adult when orderly growth is not possible, excess secretion of either or both hormones produces protein catabolic effects.

In acromegaly there is a relatively high incidence of goiter with or without hyperthyroidism. This may be due to associated involvement of the thyrotrophin producing elements of the anterior pituitary as indicated by increased TSH levels in the blood found in these patients[2].

2. GH and Insulin

GH is generally recognized as an insulin antagonist. It has long been known that injections of anterior pituitary extracts can produce diabetes in certain animal species, and all available evidence suggests that this effect is a property of GH. However, the diabetogenic effect is not observed in young growing animals in which GH produces only growth, not diabetes. What the relationship is between stimulation of the growth of tissues on one hand, and production of hyperglycemia on the other hand, is not clear.

Other evidences of existence of a pituitary diabetogenic hormone are: hypophysectomy ameliorates diabetes of the pancreatectomized dog, and injections of pituitary extracts cause the diabetes to revert to its previous severity (the Houssay phenomenon); numerous cases of human diabetes have been reported showing improvement with the onset of hypopituitarism due to tumor or atrophy of the pituitary; insulin sensitiveness is markedly increased in hypopituitarism.

The main mechanism of the diabetogenic effect of GH is believed to consist in the following sequence of events: the anti-insulin action of GH leads to hyperglycemia which constitutes an overload for the beta cells of the Langerhans islets; such overload, if long maintained, results in exhaustion atrophy of the islets and permanent diabetes. The anti-insulin effect may be due to an inhibitor of insulin which moves electrophoretically with the beta globulin in the plasma and may be responsible for the insulin resistance frequently present in patients with acromegalic diabetes[3]. In addition, it has been found that GH can probably stimulate secretion of glucagon, the hyperglycemia producing hormone of the alpha cells of the Langerhans islets[4].

The enzymatic locus of the anti-insulin effect of GH remains unknown. When it was found by Cori that anterior pituitary extracts inhibit the activity of hexokinase — the enzyme that catalyzes the first step of the metabolism of glucose, formation of glucose-6-phosphate — and that this inihibition is abolished by insulin, this was thought to be the main locus of the antagonism of the two hormones. It was soon discovered, however, that extracts of other organs (for instance, the spleen) can inhibit the hexokinase reaction, and the best purified GH preparations do not inhibit it.

Administration of GH to young animals on unrestricted food intake can lead to obesity rather than gigantism. GH is able to produce the obesity found in infants of prediabetic and diabetic mothers[5].

3. GH and the Gonads

GH is not known to have any effect on the production of sex hormones in the gonads but there are interactions between circulating GH and sex hormones. (See Chapters XII, XIV.)

4. GH and the Adrenals

GH preparations administered to human subjects have no consistent effects on the urinary excretion of 17-ketosteroids and 17-hydroxysteroids but GH prepared from human pituitaries injected into a normal subject, several hypophysectomized patients and a pituitary dwarf produced significant increase in urinary aldosterone excretion[6]. Concerning interactions between circulating GH and cortisone, see Chapter XI.

GH

may be synergistic with Thyroxine in the growing child.

antagonist of Insulin.

antagonist, in some actions, of Cortisone.

synergistic, in the pubertal growth spurt with Estrogen and Androgen.

II. THE THYROTROPIC HORMONE (TSH)

TSH of the anterior pituitary normally maintains the anatomical integrity and functional capacity of the thyroid gland. When released or administered in excess TSH can cause hypertrophy and hyperplasia of the thyroid acinar cells and an increase in the rate of thyroxine synthesis and release. The latter processes include increased iodide uptake, increased formation of thyroglobulin, decreased storage of thyroglobulin in the acini, increased rate of proteolysis of thyroglobulin and increased rate of transference of thyroxine into the circulation. The chemical mechanisms through which TSH performs these functions have not been elucidated beyond the general assumption that TSH contributes to the metabolism of the thyroid gland through some oxidative or dehydrogenating enzyme system and is in the process inactivated.

There is a balanced relationship between secretion of TSH in the anterior lobe and circulating levels of thyroxine, expressed in the concept "pituitary — thyroid axis." This interaction and its significance in thyroid and pituitary diseases will be dealt with in some detail in Chapter VII.

TSH has no interactions with other hormones and endocrine glands except *via* its effect on thyroid function. However, it can cause hypertrophy and hyperplasia of the thymus gland and lymph glands, apparently by direct action on these structures. This effect is believed to be responsible for the enlargement of the thymus and lymph glands and the lymphocytosis frequently observed in thyrotoxicosis. By promoting water retention and fat accumulation in the orbital cavity, TSH can produce exophthalmos in experimental animals, and some observers believe that the so-called malignant exophthalmos in Graves' disease may be of TSH origin.

TSH

stimulates structures of the Thyroid gland.

stimulates synthesis and release of Tyroxine.

may stimulate the Thymus and Lymph glands.

may cause exophthalmos by direct action on intra-orbital structures.

III. THE ADRENOCORTICOTROPHIC HORMONE (ACTH)

Interactions with ACTH with other glands and hormones are mediated through the adrenal cortex which ACTH stimulates to produce corticosteroids and are, therefore, similar to the interactions these corticosteroids have with other hormones. (See Chapter XI.) However, some biological effects of ACTH—including hormonal interactions — may differ from those of cortisone and cortisone analogues due to the fact that ACTH can stimulate the secretion of other steroids, especially sex steroids, as well as glucocorticoids. As a result, ACTH and cortisone may show different therapeutic results and side effects in certain diseases, and even in the same disease in different patients.

In this chapter the action of ACTH on the adrenal cortex will be reviewed along with the effect of exogenous (administered) ACTH on the pituitary and with the interrelationship between ACTH and MSH.

1. ACTH and the Adrenals

ACTH is the principal trophic hormone for the adrenal cortex; it is needed to maintain the anatomical integrity and functional capacity of the cortex. Each zone of the cortex responds to ACTH stimulation, but not equally; the zona fasciculata most, the glomerulosa least. Absence of circulating ACTH is followed by atrophy of the cortex, excess production or prolonged administration is followed by hypertrophy, occasionally by ademnoma. principal response in steroid production occurs in *hydrocortisone* and *corticosterone*, but there is strong evidence that androgen, estrogen, and progesterone of their precursors, too, participate in the response[8].

Thus, concerning *androgen* production, ACTH administration is followed by a prompt rise of urinary 17-ketosteroids; as this does not occur in Addison's disease, and since it is known that ACTH does not stimulate the production of gonado-

trophin in the pituitary and has no direct effect on gonadal androgen production, it was concluded that androgenic substances or their precursors can be produced in response to ACTH. This is also suggested by the high incidence of acne and of excess growth of body hair in patients on prolonged ACTH therapy.

(On the other hand, careful studies by Moore[9] found no convincing evidence that ACTH stimulates androgen secretion by the adrenals or that the adrenals under normal conditions secrete androgen, and raised the question whether the pathological physiology of virilising adrenal tumors may involve *atypical* synthesis of steroids that account for the clinical symptoms. Also, it was found that ACTH does not produce androgenic effects in castrated rats; and cortisone administration, too, can be followed by acne and hypertrichosis[10].)

Concerning the secretion of *estrogen* by the adrenal cortex in response to ACTH it has been shown that ovariectomized but otherwise normal women receiving large doses of ACTH excrete increased amounts of estrogen in the urine; as there is no increase in estrogen excretion when they receive large doses of hydrocortisone, it was concluded that ACTH can stimulate adrenocortical estrogen production[11].

There s evidence that ACTH administration is capable of affecting *aldosterone* excretion, but ACTH is not the primary or main regulatory factor in aldosterone production. The aldosterone response to ACTH may be masked by a homeostatic regulatory mechanism that responds to increased extracellular fluid volume and is independent of ACTH[12].

Concerning the chemical mechanism of the ACTH effect on the synthesis of hydrocortisone and corticosterone, there is evidence that the main effect occurs at some stage of the conversion of cholesterol to a C-21 precursor such as progesterone, promoting the degradation of the cholesterol side chain by an adrenocortical enzyme system. This, however, may not be the only locus, in that ACTH can enhance subsequent transformations[13]. The intimate details of the mode of action of ACTH are yet to be learned.

ACTH given during prolonged cortisone therapy, or during the first few days after cortisone had been discontinued, is evidently ineffectual because the adrenal cortex had undergone atrophy and is incapable of adequately responding to ACTH. (See Point 1, Chapter XI.) On the other hand, repeated trauma, surgical stress, chronic illness instead of exhausting the cortex, produce hypertrophy with exaggerated response to ACTH as shown by greater than normal increase of 17-hydroxysteroids in the plasma and urine during and after standard ACTH infusion; the more severe the trauma the more marked and prolonged is this response. In chronically ill patients the response to ACTH is 1.5 to 2 times greater than in normal persons. The postoperative elevation of the plasma corticoids starts within an hour and lasts 1 to 3 days; with complications such as infection, it lasts longer; in severe burns it lasts more than a week. When the plasma steroids return to normal, the response to infused ACTH, too, returns to normal[14].

Intramuscular injection or intravenous infusion of ACTH followed by eosinophil counts and/or assays of plasma or urinary corticosteroids serve as important tests in the diagnosis of adrenocortical insufficiency and hyperactivity. In *primary* Addison's disease the patient shows poor response or no response to administered ACTH, while in *secondary* adrenocortical insufficiency—due to pituitary failure—the response to ACTH, after repeated injections, is normal. Cases of "partial" Addison's disease have been described where the serum electrolytes and corticoids are within normal range but there is poor response to ACTH, indicating that the adrenal cortex is functioning at maximum capacity having no reserve that would be needed in stress situations.

In Cushing's syndrome due to adrenocortical hyperplasia the response to intravenously administered ACTH is usually excessive with marked elevation of the plasma 17-hydroxysteroid level and increased urinary excretion of these steroids; the urinary 17-ketosteroids remain within normal range[16]. In Cushing's syndrome due to adrenocortical car-

cinoma the response to ACTH is less than normal; with benign tumors of the cortex the response may be either normal or increased. Accordingly, in some cases, this test can furnish valuable aid in differential diagnosis. In adrenocortical hyperplasia due to prolonged ACTH administration the adrenocortical response to ACTH is usually normal[17].

In hyperaldosteronism due to adrenocortical hyperplasia intravenous ACTH administration is reported to be followed by excessive elevation of plasma and urinary 17-hydroxy corticosteroids, and it has been suggested that this test might be utilized to distinguish between that disease and hyperaldosteronism due to adrenocortical tumor.

2. ACTH (Exogenous, administered) and Release of Endogenous ACTH

Prolonged administration of ACTH is believed to inhibit release of ACTH from the anterior pituitary. This is indicated by a) the degranulation of basophilic cells in the anterior loge with development of Crook's hyalin cytoplasmic changes in experimental animals after prolonged administration of ACTH, and b) the development, in experimental animals as well as in man, of signs of adrenal insufficiency with low urinary 17-ketosteroids and 17-hydroxysteroids after withdrawal of prolonged ACTH administration. Recovery of normal ACTH production is slow, and therefore withdrawal of ACTH therapy must be very gradual over a period of several weeks to avoid development of potentially dangerous adrenal insufficiency during that period.

The mechanism of the inhibition of pituitary ACTH production by exogenous (administered) ACTH is believed to be as follows: the administered excess ACTH provokes excess release of corticosteroids from the adrenal cortex and these in turn inhibit endogenous ACTH production in the pituitary.

3. ACTH and the Melanocyte Stimulating Hormone (MSH)

Soon after ACTH became available for clinical use, numerous cases of Addison-like hyperpigmentation were reported in patients on prolonged ACTH therapy; in some patients new pigmented nevi appeared or nevi already present became darker. It was found that small quantities of added ACTH could darken frog skin *in vitro*. Much debate developed about the question whether ACTH and MSH are identical, and the hyperpigmentation in primary Addison's disease is due to ACTH (which is known to be released in excessive amounts in that disease). It is believed at present that ACTH and MSH are chemically closely related but not identical (as are oxytocin and pitressin); that the hyperpigmentation of Addison's disease is due to increased amounts of MSH in that disease, and the hyperpigmentation seen in ACTH-treated patients is due to MSH present as a contaminant in the ACTH preparations. (See also Chapter V.)

ACTH
stimulates the Adrenal Cortex.
stimulates in the adrenal cortex synthesis and release of
Glucocorticoids
Androgens
Estrogens
(Aldersterone)
inhibits release of ACTH from Anterior Pituitary has interactions with other glands and hormones similar to the interactions of Cortisone.

IV. THE GONADOTROPHINS

The anterior pituitary produces three different gonadotrophic hormones: the follicle stimulating hormone (FSH), the luteinizing hormone (LH) and the luteotrophic hormone (LTH). LH is identical with the interstitial cell stimulating hormone (ICSH) in the male; the role of LTH in the male has not been definitely established.

1. Gonadotrophins (administered, exogenous) and Release of Endogenous Gonadotrophins

Parenteral administration of gonoadotrophin causes suppression of endogenous gonadotrophin formation or release from the pituitary in animals with intact glands but not in castrated animals. This indicates that the gonads stimulated by the administered gonadotrophin produce excess amounts of gonadal hormones which, in turn, inhibit endogenous gonadotrophin release[18].

2. Gonadotrophins and the Ovary

FSH has mostly morphologic effects

on the ovary, stimulating the growth of the Graafian follicle, but does not significantly stimulate production of the ovarian hormones. LH induces the consecutive phases of follicular development, ovulation, formation of luteal tissue, and promotes secretion of estrogen in the theca cells of the follicle as well as secretion of progesterone in the corpus luteum. FSH and LH augment each other's effects on the ovary. LTH helps to maintain the functional capacity of the corpus luteum after FSH and LH had acted on it; it is believed to be identical with Prolactin which stimulates secretion of milk in the mammary glands after they had been conditioned by the ovarian hormones.

Observations on the hypophysectomized female rat indicate that the early phases of oogenesis and follicle development proceed without need of gonadotrophic stimulation; however, none of the follicles mature or luteinize. In the adult female, hypophysectomy is followed by atrophy of all ovarian structures which can be prevented by administration of purified gonadotrophic preparations.

The production of these trophic hormones in the anterior pituitary is subject to regulation by various factors, predominantly the plasma levels of circulating ovarian hormones, and possibly also the rate of utilization of the gonadotrophins by the ovaries. This regulation is expressed in the term "pituitary-ovarian axis" and will be further discussed in the chapters on gonadal hormones. Here only a specific regulation involved in the menstrual cycle will be described. This is usually summarized as follows:

Release of FSH, acting synergistically with a small amount of LH, stimulates follicle development in the ovary. Activity of the cells of the theca interna results in secretion of estrogens which in turn induce secretion of follicular fluid by the granulosa cells and sensitize the follicle to FSH. The estrogens that were produced enter the blood stream and act on the anterior lobe of the pituitary, inhibiting further FSH production, while secretion of LH and LTH is increased. LH acts on the sensitized follicle causing its rupture (with the release of the ovum), stimulates development of the corpus luteum in the ruptured follicle, and LTH stimulates secretion of progesterone by the corpus luteum. The progesterone thus produced soon inhibits further secretion of LH in the anterior lobe, resulting in rapid decline of progesterone and estrogen secretion by the corpus luteum; the uterine mucosa is thus deprived of hormonal support, the mucosal circulation breaks down ending in menstrual bleeding. The low level of estrogen and progesterone circulating in the blood at this time allows renewed secretion of FSH and LH in the anterior lobe, and a new cycle begins.

One must realize though that the *details* of such precisely timed and quantitative interplay between the pituitary gonadotrophins and the ovarian hormones are somewhat speculative. Actual gonadotrophin assays (FSH plus LH) in the urine during the menstrual cycle in normal woman showed that no pattern could be reproduced from cycle to cycle or from subject to subject; minimal amounts of gonadotrophins were found less frequently at midcycle but with equal frequency during either the follicular or the luteal phase. Better reproducibility of the pattern of excretion and a sharper peak at midcycle resulted when a method was used that presumably measures only LH[19].

The composition of gonadotrophins in pituitary extracts is inconsistent, the ratio FSH/LH varying in extracts made from different species of animals and with different methods of purification. In man all assays of gonadotrophins are based on urinary gonadotrophins, usually designated as FSH, but there is evidence that the urinary residues usually prepared in the assays contain also the biological activities of LH; it has not been proved that these hormones can be separated from the urine[19]. In two other types of gonadotrophic preparations, chorionic gonadotrophin (CG) and pregnant mare serum (PMSG), the ratio of FSH/LH is more constant, CG containing much more LH than FSH, and PMSG containing more FSH than LH. The large amounts of gonadotrophins excreted in pregnancy are of chorio-placental, not pituitary, ori-

gin; their urinary excretion provides the basis for several "pregnancy tests." Their principal function is to stimulate the corpus luteum of pregnancy and thereby provide for continued secretion of estrogen and progestrone by that organ. Certain tumors derived from chorionic tissue, i.e., chorionepithelioma in women or in men and hydatidiform mole in women may produce very large amounts of CG, and their diagnosis may be facilitated by assays of gonadotrophin in the urine.

Excess production of gonadotrophins in the anterior pituitary (primary hypergonadotrophism) manifested by precocious isosexual puberty or, in acromegaly by hypertrophy of the genital tract, is rare. Most acromegalic women show amenorrhea and sclerotic ovaries indicating primary hypogonadotrophism as a result of atrophy of the gonadotrophin-producing (basophilic) cells in the anterior lobe from the pressure of the eosinophilic tumor.

Primary hypogonadotrophism is common in all forms of primary hypopituitarism in women. About secondary hyper- and hypogonadotrophism resulting from hypo- and hyperestrinism, see Chapter XII.

3. Gonadotrophins and the Testis

In the male, FSH stimulates the seminiferous tubules and promotes spermatogenesis, while LH (ICSH) stimulates the Leydig cells and promotes secretion of androgen (and estrogen) by the testicles. Primary excessive production of gonadotrophins is rare as a cause of precocious puberty in the male, but occurs not infrequently in acromegalics who may show, especially early in the course of the disease, increased libido and potency, hypertrophy of the genitals and excessive hirsutes. Failure of gonadotrophin production is a common and early feature of primary hypopituitarism. For the secondary forms of hyper- and hypogonadotrophism, due to absence or excess of circulating androgen, see Chapter XIV.

4. Gonadotrophins and the Adrenals

In view of the fact that the adrenal cortex can secrete androgen and estrogen the question arose whether these secretions — presumed to be under the control of ACTH — are controlled also by gonadotrophins, especially LH. There is considerable evidence both for and against a stimulating effect of chorionic gonadotrophin on adrenocortical activity; however, much of the evidence supporting existence of such trophic effect is indirect and inconclusive. Recently, Hibbitt and his associates[20] found that human chorionic gonadotrophin administered to men intravenously in large doses does not lead to increased urinary excretion of total 17-hydroxysteroids; furthermore, there is obvious difference between the pattern of increased 17-ketosteroid excretion following these injections and the pattern following ACTH administration. They concluded from these data that human chorionic gonadotrophin stimulates only the gonadal, not the adrenocortical, tissue. Another point that supports this view refers to the experience that the adrenocortical androgenic tissue and the pituitary gonadotrophins do not have the typical reciprocal relationship that exists between testicular tissue and pituitary gonadotrophins. To illustrate this Hibbitt quotes studies on a case of adrenogenital syndrome in a 75 year- old woman who had high levels of both urinary 17-ketosteroids and urinary gonadotrophins. If the reciprocal relationship between adrenal androgens and the pituitary were similar to that between gonadal androgens and the pituitary, one would expect in this patient inhibition of the release of pituitary gonadotrophins (in spite of the usual post-menopausal increase) by the elevated adrenal androgens. Even more impressive is the experience that chorionic gonadotrophin does not produce increased urinary 17-keosteroids in gonadectomized patients.

No data are available indicating direct effects of gonadotrophins on other glands.

FSH
stimulates follicle development in Ovary
synergistic with LH in stimulating secretion of Estrogen and Progesterone in Ovary
stimulates seminiferous tubules and spermatogenesis in Testis
inhibits release of endogenous FSH

LH (ICSH)
stimulates luteinization of Corpus Luteum and secretion of Estrogen and Progesterone
synergestic with PSH in actions on Ovary
stimulates Leydig cells and secretion of Androgen in Testis
inhibits release of endogenous LH

LTH (Prolactin)
maintains function of Corpus Luteum
synergistic with LH in secretion of Progesterone
stimulates milk secretion (after mammary glands had been conditioned by Estrogen and Progesterone)

V. THE MELANOCYTE STIMULATING HORMONE (MSH)

MSH is a relatively small polypeptide produced in the intermediate lobe of the pituitary. It is chemically closely related to ACTH, having a very similar composition. In coldblooded vertebrates MSH acts on the chromatophores of the skin causing dispersion of their pigment granules. The mechanism of action of MSH in mammals is more complex, and is the subject of various theories[21]. The visible effect is increased melanin pigmentation.

MSH is not known to have any effect on the production or release of other hormones, but the release and effect of MSH is subject to control by other hormones, especially cortisone and epinephrine. (See Chapters IX and X.) The thyroid and gonadal hormones, too, have been shown to influence melanin pigmentation in animals and man, but it is believed that this occurs by independent peripheral action on the melanocytes with only permissive action, or without any involvement, of MSH.

In the rabbit intradermal injection of thyroxine causes increased pigmentation in the injected area. In man, hyperthyroidism is frequently associated by increased general pigmentation, while myxedematous patients are usually pale. Local administrations of estrogen to the nipples produce increased pigmentation in the area; and the deep pigmentations around the genitalia and linea alba in pregnancy are believed to be estrogenic effects due to stimulation of the melanocytes in those areas by the greatly increased amounts of circulating estrogens. However, in the hyperpigmentations of pregnancy MSH may also be actively involved since it has been demonstrated that MSH levels in the blood and urine are elevated during pregnancy; they rapidly decline to normal levels within a few days after delivery[22]. Androgen and progesterone, too, have slight pigmentogenic effects by direct stimulation of the melanocytes. The hyperpigmentation frequently accompanying prolonged ACTH therapy is probably due to the presence of MSH in the ACTH preparations as a contaminant.

MSH
release inhibited by Cortisone (see Chapter XI)
release stimulated by Epinephrine (see Chapter X)
effect antagonized by Epinephrine (see Chapter X)

VI. THE HORMONES OF THE POSTERIOR PITUITARY

Normally, the posterior pituitary hormones—pitressin (believed to be identical with the antidiuretic hormone, ADH) and oxytocin—do not directly influence hormone production in other glands[23] except, possibly, aldosterone secretion in the adrenal cortex[24]. But actions of the posterior pituitary hormones may be intimately involved, synergistically or antagonistically, with actions of certain other hormones[25].

1. **Oxytocin, Estrogen and Progesterone,** see Chapter XIII, Point 2.
2. **ADH and Anterior Pituitary hormones**

Certain anterior pituitary hormones are antagonistic to ADH as suggested by the fact that permanent diabetes insipidus—which follows destruction of any of the tissues that secrete, transport or store ADH, i.e., hypothalamic nuclei, stalk and posterior pituitary—rarely develops after total hypophysectomy. This is explained by pointing out that with total hypophysectomy the antidiuretic hormone as well as the "diuretic" hormones of the anterior lobe are removed. At least two anterior lobe hormones, TSH and ACTH *via* trophic effects on their target glands participate in this activity and may be regarded as diuretic antagonists of ADH. Recent experience with patients who had been hypophysectomized for metastatic breast cancer have shown that severe diabetes insipidus frequently develops if the infundibular stalk had been divided "high," i.e., near to its hypothalamic end;

this results in traumatization of the base of the hypothalamus and is followed by *complete* deficiency of ADH. Furthermore, in these patients who are maintained on cortisone temporary withdrawal of cortisone does not ameliorate the polyuria. Accordingly, in complete abscence of ADH, diabetes insipidus can develop regardless of the abscence or presence of anterior pituitary activity. Apparently, then, in the regulation of water diuresis the capacity of anterior pituitary hormones to antagonize ADH is limited and plays but a minor role[98].

3. ADH and Thyroxine

As mentioned in the previous paragraph, thyrotrophin exerts antagonistic action to ADH by stimulating thyroxine production in the thyroid gland. Thyroid feeding produces polyuria after total hypophysectomy, but thyrotropin has no diuretic effect after thyroidectomy. Hyperthyroid patients fail to show decrease of diuresis after administration of large amounts of ADH (pitressin); and the polyuria in diabetes insipidus is frequently ameliorated by thyroidectomy, returning to its previous severity when thyroid is administered. All this clearly demonstrates antagonism between ADH and thyroxine.

4. ADH and Adrenocortical Hormones

Generally speaking, the actions of ADH are antagonistic to those actions of adrenocortical steroids which are concerned in the control of the renal excretion of water and electrolytes. These interactions are parts of a rather complicated mechanism in which ADH inhibits renal reabsorption of sodium chloride and stimulates reabsorption of water, while the steroids stimulate reabsorption of sodium and inhibit reabsorption of water. Glucocorticoids having oxygen atoms at carbons 3, 11, 17, 20 and 21 such as cortisone and hydrocortisone are more capable of inhibiting water reabsorption than stimulating sodium reabsorption, and are therefore antagonists of ADH. By virtue of this antagonism, administration of these corticosteroids can produce polyuria after total hypophysectomy; and lack of these steroids may be responsible for the failure of water diuresis, after water leading, in Addison's disease as shown by the Kepler-Robinson test. Desoxycorticosterone, aldosterone and the adrenal sex hormones are more effective in causing salt retention (than causing water diuresis) and therefore may produce considerable water retention. Whether water retention follows the salt retention (as the body tends to retain water while conserving salt) or salt retention causes increased ADH release from the posterior pituitary which in turn would cause water retention is not known. In any case, these corticosteroids may be regarded as more or less synergistic with ADH.

5. ADH and Gonadal Hormones

Androgens and estrogens may be regarded as synergistic with ADH in the sense stated above whether they are of gonadal or adrenocortical origin. It may be added that the ADH level in the blood is higher than normal in pregnancy, but the mechanisms through which this increase is brought about is not known[26].

OXYTOCIN
- *effect on uterus may be potentiated by Estrogen (see Chapter XIII)*
- *effect on uterus may be antagonized by Progesterone (see Chapter XIII)*

ADH (PITRESSIN)
- *antidiuretic effect antagonized by ACTH, TSH, Thyroxine, Cortisone*
- *synergistic with antidiueretic effects of Aldosterone, Androgens, Estrogens*

VII. THYROXINE

1. Thyroxine and the Thyrotrophic Hormone (TSH)

It is well established that circulating thyroxine (and triiodothyronin) inhibits the release of TSH from the pituitary. Circulating thyroxine is mostly bound to alpha globulin in the serum but some of it is present in free form, and it appears likely that its actions, including action on the pituitary, are a function of the free fraction.[27] It is believed that the inhibitory effect on TSH release is mediated via thyroxin-receptor cells in the hypothalamus through the portal circulation in the hypophyseal stalk. It is possible, though, that one or more of the metabolic products of the peripheral thyroxine action rather than the circulating thyroxine level itself is the real regulatory factor for the release of TSH.

This is suggested by the fact that administration of 2, 4-dinitrophenol — a powerful stimulant of tissue oxidation — depresses TSH output in the rat, rabbit,[28] and man[29] as effectively as does thyroxine. Whatever the mechanism is, the result is an automatic feed-back regulation in the 'pituitary-thyroid axis' in which high levels of circulating thyroxine and/or its metabolites lead to inhibition of TSH release resulting in lowered levels of thyroxine release.

In primary myxedema TSH activity of the blood and urine is much higher than normal indicating increased release of TSH from the pituitary as a result of the low level of circulating thyroxine. Assays of TSH can be used to differentiate primary myxedema from secondary (pituitarigenic) myxedema in which the blood and urinary levels of TSH are low or zero.

In thyrotoxicosis TSH activity of the blood and urine is low or absent. This has been interpreted by some as evidence against a pituitary pathogenesis of thyrotoxicosis. However, advocates of such pathogenesis — claiming that the primary disturbance in thyrotoxicosis consists in increased production of TSH — point out that tissue slices of toxic goiter show greatly increased capacity to inactivate TSH in vitro, which would explain the low levels of TSH in the blood in Graves' disease even if large amounts of TSH were released from the pituitary. Observations concerning the effect of administered thyroid hormone upon the thyroidal radioiodide uptake were brought into this controversy: the effect is more or less complete suppression of uptake in normal persons but no effect in hyperthyroidism. More recently, the effect of administered thyroxine upon the thyroidal secretion rate (release rate) was studied with the result that normal subjects showed prompt and nearly complete inhibition of release, whereas in all hyperthyroid patients only a slight alteration in hormonal secretion rate occurred in spite of massive doses of thyroxine[30]. While these findings appear to be strongly in favor of primary pituitary disturbance as a common cause of thyrotoxicosis, the development of toxic goiter in patients years after hypophysectomy, (showing total pituitary insufficiency) indicates that thyrotoxicosis can develop without pituitary thyrotropic hyperactivity[99].

2. **Thyroxine and GH**

It is not known whether thyroxine can influence production or release of GH. There is a certain synergism in the promotion of growth between the two circulating hormones as mentioned in chapter I.

3. **Thyroxine and ACTH**

Pituitaries of experimental animals with altered states of thyroid function have been found to contain normal amounts of ACTH.[31] In human thyroid diseases adrenocortical functions may be abnormal — as will be pointed out below —but these appear to be due to action of thyroxine on the adrenals rather than to effects on ACTH release.

4. **Thyroxine and Gonadotrophins**

There is much evidence, experimental as well as clinical, that the thyroid gland may influence gonadal function but it is not known whether this occurs via gonadotrophins of the pituitary or by direct effect on the gonads (see point 8). Controlled assays of gonadotrophins in the blood and urine in thyroid disorders are not available. Desiccated thyroid added to the food of cows and hens is widely used by farmers to increase milk and egg production; this is probably effected by stimulating pituitary gonadotrophin activity.

5. **Thyroxine and the Thyroid Gland**

There is evidence of *direct* inhibitory action of thyroxine on the thyroid gland as shown in hypophysectomized animals and patients[32], aside of the indirect inhibitory effect *via* TSH production outlined in point 1 in this chapter.

6. **Thyroxine and Insulin**

In the homeostasis of blood sugar thyroxine is one of the (minor) factors that tend to elevate blood sugar, and therefore it may be regarded as an insulin antagonist. However, total thyroidectomy in euthyroid persons without

diabetes mellitus causes no important change in fasting blood sugar nor in sensitiveness to insulin. Most patients with thyrotoxicosis show impaired glucose tolerance, believed to be caused by the high level of circulating thyroxine as well as by thiamin deficiency, hepatic disease and depletion of the glycogen stores commonly present in that disease; all this may lead to excessive demands on the islets, aggravate an inherited disposition for diabetes and account for the fact that the incidence of diabetes in patients who have or had thyrotoxicosis is twice that in the general population. In some diabetics with thyrotoxicosis great improvement of the diabetes follows thyroidectomy. Diabetes occasionally occurs as an incidental complication of myxedema; treatment of the myxedema with thyroid causes marked increase of the severity of diabetes in these patients.

7. **Thyroxin and the Adrenals**

The circulating level of thyroxine markedly influences the hormone secretion and the effects of the circulating hormones of the adrenal cortex but the interactions are complex and cannot be described in simple terms of stimulation or inhibition. This is strikingly illustrated by observations showing seemingly identical alterations of adrenal functions in both hypothyroidism and hyperthyroidism.

In *myxedema* the urinary excretion of 17-ketosteroids and 17-hydroxysteroids is abnormally low, and there is decreased hair growth in the axillary and pubic regions, indicating decreased adrenocortical functions. This has been described as 'myxedema of the adrenals' which can be restored to normal by appropriate (cautious) thyroid therapy. Apparently, the adrenal cortex, like other tissues of the body, operates at a lower than normal metabolic level in the state of hypothyroidism. Recently, however, it has been reported that the 17-hydroxysteroid levels in the *plasma* were normal in myxedema, and administration of ACTH provoked normal eosinophil response suggesting that a diminished rate of utilization of the corticosteroids in the peripheral tissues (in addition to diminished rate of production in the adrenal cortex) occurs in myxedema.[33] Administration of thyroid in larger than minimal doses to patients with myxedema may produce manifestations of adrenal insufficiency; this is usually explained by assuming that the low levels of corticosteroids prevailing in myxedema may be sufficient for the needs of the body at the low levels of metabolism but became inadequate at the higher levels of metabolism producd by thyroid administration. Similar reasoning may be applied to explain the observation that administration of thyroid extract to patients suffering from Addison's disease may percipitate an Addisonian crisis.

In *thyrotoxicosis* there is decrease in the urinary excretion of 17-keto as well as 17-hydroxy steroids and, frequently, loss of axillary and pubic hair — the same changes found in myxedema. Thyrotoxicosis may be considered as a state of 'alarm reaction' and it is known that in alarm reactions the adrenal androgen activity is decreased resulting in low 17-ketosteroid excretion. The low 17-hydroxysteroid levels, on the other hand, may be due to increased utilization of these steroids in view of the high level of general tissue metabolism. The result is a state of 'relative adrenocortical insufficiency' even though the adrenals may be more active than normally. A hightened level of adrenocortical activity is indicated also by the relative insensitivity to stimulation by ACTH found in thyrotoxicosis. However, long duration of thyrotoxicosis may result in depletion of adrenal reserve and capacity.[34]

8. **Thyroxine and the Gonads**

In hypothyroidism there may be cryptorchism, decrease in libido and fertility in both sexes; impotence in males; menorrhagia is frequent, but in severe myxedema amenorrhea may occur. All these are probably sequelae of low levels of metabolism in the gonadal and/or pituitary gonadotrophic cells, and may be relieved by thyroid therapy. In thyrotoxicosis the menstruation tends to be scant and infrequent, probably as a result of toxic levels of metabolism in the

ovarian structures. Some women report breast enlargement at the onset of thyrotoxicosis, and in male hyperthyroids gynecomastia develops occasionally; the mechanism if this effect is not known — it might be connected with the decreased adrenal androgenic activity mentioned above.

THYROXINE
inhibits release of TSH from pituitary
synergistic with GH in the growth of children
may stimulate Pituitary gonadotrophic activity
can inhibit the Thyroid by direct effect
may antagonize Insulin
can stimulate release and utilization of Adrenal Corticoids
may stimulate Gonadal activity

VIII. THE PARATHYROIDS

Most of the evidence favors the opinion that the parathyroids are not under the direct influence of the anterior pituitary by way of a parathyrotrophic hormone; neither has the parathyroid hormone any specific effect on the pituitary. However, certain clinical observations have repeatedly called attention to possible relationships with other glands, especially the adrenals. Thus, hypoparathyroidism was found associated with *Addison's disease* in a few cases[35] and, rarely, parathyroid tumors were found in *Cushing's syndrome*. Osteoporosis is common in Cushing's syndrome, occasionally with hypercalciuria, nephrolithiasis and elevated serum alkaline phosphatase — findings characteristic for hyperparathyroidism; however the histology of the bony changes is quite different in the two diseases, and in Cushing's syndrome the serum alkaline phosphatase never attains the level seen in hyperparathyroidism. The osteoporosis in Cushing's syndrome is believed to be due to deficient bone matrix formation resulting from decreased activity of the osteoblasts, in contrast with the decalcification resulting from the phosphate diuresis in hyperparathyroidism.

Rarely, parathyroid tumors were reported associated with multiple *pancreatic* islet cell tumors with or without *pituitary* tumors[36]. Whether all these associations are purely co-incidental or expressive of some hidden relationship is not known.

Some sort of synergism between parathyroid hormone and *estrogen* has been suggested to explain the observation that hypoparathyroidism following thyroidectomy may remain latent or compensated in premenopausal women for years, only to become manifest after the menopause when it can be relieved by estrogen administration. Soffer in reviewing the available data concluded that "it is difficult to feel that there is any significant relationship" between the parathyroids and the adrenals or other glands.[37]

IX. INSULIN

Insulin production in the beta cells of the Langerhans islets is regulated by the prevailing blood sugar, high levels of blood sugar stimulating insulin production and release, low levels inhibiting it. Release of insulin is not influenced by anterior pituitary or other hormones except in so far as they affect the blood sugar level. Thus, GH, ACTH, TSH, *thyroxine*, *epinephrine*, *cortisone* and *glucagon* are insulin antagonists by virtue of their capacity to elevate the blood sugar level and are, in turn, antagonized in that process by insulin; together they constitute the main elements of the blood sugar regulatory mechanism.

Growth hormone and insulin may be mutually synergistic under certain conditions (see chapter I). Hypophysectomized young rats on nutritious diet show better growth on insulin plus GH administration than on either hormone alone.[38] Epinephrin and glucagon are, while antagonizing insulin by contributing to blood sugar at the expense of liver glycogen, synergistic with insulin in providing the very substrate the utilization of which is enhanced by insulin.

Insulin is not known to have any effect on the production or release of other hormones except *epinephrine*. Intravenously administered insulin causes marked increase in the secretion of epinephrine — but not of nor-epinephrine — in the adrenal medulla, presumably as a compensatory response to the hypoglycemia produced by the insulin.[39] Thus, insulin has a dual interaction with epinephrine: it can stimulate the pro-

duction and antagonize the action of epinephrine.

INSULIN
may be synergistic with GH in the growing child
stimulates release of Epinephrine from the Adrenal Medulla
antagonist, in the regulation of blood sugar, to GH, TSH, ACTH, Thyroxine, Epinephrine, Glucagon

X. EPINEPHRINE

1. Epinephrine and ACTH

Long and his associates[40] found that injection of epinephrine into the rat causes decrease in the ascorbic acid and cholesterol content of the adrenal cortex to an extent and within the time relationships of that following administration of ACTH. Since epinephrine is ineffective in the hypophysectomized rat, they concluded that in the normal animal it acts on the anterior pituitary augmenting the secretion of ACTH. Epinephrine injection in the dog increases five to tenfold the plasma content of cortical hormones in the adrenal vein.[41]

Epinephrine injection to normal persons is followed by a fall of the blood eosinophils greater than 50% in 4 hours. Since it was known that ACTH injection has a similar effect, it was postulated that epinephrine causes a fall of the eosinophil count by way of stimulating release of ACTH. However, it was later found that similar eosinophil response to epinephrine occurs in adrenalectomized patients maintained on constant amounts of cortisone, which indicates that this particular effect of epinephrine does not depend on stimulation of ACTH production.

2. Epinephrine and TSH

Epinephrine can stimulate the *release* of TSH[42] and, in addition, augment the thyroid stimulating *action* of TSH.

3. Epinephrine and Gonadotrophins

There is evidence that epinephrine can stimulate the release of gonadotrophins in laboratory animals.[44]

4. Epinphrine and MSH

There are two seemingly opposite actions of epinephrine on melanin pigmentation in the frog, according to Lerner.[45] One is to stimulate *release* of MSH in the pituitary, the other is to block the *action* of MSH on the melanocyte (in the isolated skin). However, there is no direct evidence that epinephrine affects pigmentation in man.

Thus, epinephrine can stimulate the release of ACTH, TSH, and, probably, FSH and MSH. It is believed that these effects on the pituitary are mediated *via* hypothalamic centers through the so-called portal circulation in the stalk.

5. Epinephrine and Insulin

One of the principal actions of administered epinephrine is its capacity to produce hyperglycemia by inducing glycogenolysis in the liver. It was assumed that epinephrine as an insulin antagonist plays a dominant role in blood sugar homeostasis, especially after insulin-induced hypoglycemia. Such role of epinephrine, however, became doubtful when it was found that in bilaterally adrenalectomized patients recovery from insulin-induced hypoglycemia proceeded normally, indicating that in the absence of adrenals other insulin antagonists can effectively take over[46].

Antagonism of epinephrine to insulin, of a different nature, is suggested by Groen and associates[47] who found that the stimulation of glucose utilization by insulin in the isolated rat diaphragm is strongly suppressed by very small amounts of added epinephrine or norepinephrine; this is not due to interference with the binding of insulin by the diaphragm tissue.

EPINEPHRINE
stimulates release of ACTH, TSH, FSH, MSH from Pituitary
synergistic with the thyroid stimulating action of TSH
antagonist of Insulin in blood sugar regulation
blocks action of MSH on the Melanocyte

XI. CORTISONE AND CORTISONE ANALOGUES

1. Cortisone and ACTH

Administration of large amounts of cortisone results in adreno-cortical atrophy which may be prevented by simultaneous administration of ACTH. This and many other observations prove that circulating cortisone can inhibit the production of ACTH in the anterior pitui-

tary; this is one of the best documented hormonal interrelations. By way of this feed-back mechanism circulating cortisone can regulate its own endogenous production in the adrenal cortex.

The adreno-cortical atrophy during and following cortisone administration is of great practical importance. Severe adrenal insufficiency and even fatal adrenal crisis can occur if, after prolonged administration, cortisone is suddenly discontinued. The danger is especially acute during the first week after cortisone is discontinued; but complete restoration of the adrenal cortex capable of coping with severe stress situations such as major surgery may not take place for many months. However, there is no permanent failure of adrenocortical response to endogenous or exogenous ACTH even after years of cortisone treatment[15]. There has been some controversy concerning the need for intermittent administration of ACTH during cortisone therapy in order to prevent adrenal atrophy. Most clinicians with experience in cortisone therapy do not use ACTH during and after cortisone therapy but take care to discontinue cortisone very gradually over a number of weeks, and administer cortisone when any severe stress situation arises, even a year or two later.

The inhibitory effect of cortisone on ACTH release has been utilized in the diagnosis and therapy of certain adrenal diseases. Thus, cortisone administration has been recommended to facilitate differential diagnosis in certain adrenocortical diseases (adreno-genital syndrome; Cushing's syndrome) between that due to hyperplasia and that due to tumor. Large dosage of cortisone suppresses endogenous ACTH production leading to decrease of the 17-ketosteroids in the urine in cases of hyperplasia; in cases of tumor (which is not dependent on ACTH) no change in the urinary 17-ketosteroids would occur. However, these tests are not wholly reliable.

Cortisone therapy has been very useful in certain types of adrenal hyperplasia, such as congenital adrenal hyperplasia, prepubertal adrenal hyperplasia and the Stein-Leventhal syndrome. Congenital adrenal hyperplasia, manifested by pseudo-hermaphroditism in female infants is presumably due to an 'inborn error' in the synthesis of hydrocortisone. A precursor, 17-hydroxyprogesterone is normally converted to hydrocortisone by hydroxylation at C_{11} and C_{21}. This enzymatic process is blocked in congenital adrenal hyperplasia with the result that less hydrocortisone is produced while the accumulating precursors are diverted into excess androgens. The low level of circulating hydrocortisone allows excess production of ACTH in the anterior pituitary which, in turn, causes adrenocortical hyperplasia with further excess production of 17-hydroxyprogresterone[48]. Administration of cortisone, by inhibiting ACTH production, inhibits the adrenocortical hyperplasia and the accumulation of hydrocortisone precursors and androgens with the result that the virilizing effects are ameliorated.

The Stein-Leventhal syndrome — hyperthecosis, small-cystic ovaries, amenorrhea, hypertrichosis and, frequently, obesity, with high-normal or slightly elevated urinary excretion of 17-ketosteroids and elevated excretion of pregnanediol and pregnanetriol — is thought to be of adrenocortical origin, and this is supported by the observation that cortisone administration can suppress the urinary excretion of these steroid metabolites in these patients, frequently restores normal menstruation and occasionally relieves the hypertrichosis.[49]

Administration of cortisone in man *via* inhibition of ACTH release or otherwise has normally no significant effect on the excretion of aldosterone.[50] On the other hand, administration of prednisone has been reported to lower the urinary aldosterone excretion in patients with hepatic cirrhosis — whose aldosterone excretion is usually higher than normal — resulting in sodium diuresis.[51]

The question arose whether cortisone aside of its effect on the release of ACTH can also have a *direct* effect on the adrenal cortex. This has been investigated in hypophysectomized animals and human patients maintained on constant amounts of ACTH. No consistent results

were obtained, and the question remains open at present.

2. **Cortisone and GH**

There is no evidence that cortisone has any effect on the production or release of GH, but under certain conditions cortisone can antagonize the somatotrophic actions of GH. Administered in large amounts to experimental animals cortisone inhibits normal growth as well as the growth effect of GH preparations.[52] This might be due to the general catabolic effects of cortisone as against the general anabolic effects of GH. Other manifestations of this antagonism may be recognized in the fact that children with Cushing's syndrome—who carry excess glusocorticoids in their body —are usually retarded in growth, and children with congenital adrenocortical hyperplasia show slower growth while under cortisone treatment. The two hormones also antagonize each other's action on capillary resistance in the rat and dog, cortisone increasing it while GH depresses it.[53] On the other hand, the two hormones are synergistic in their opposition to insulin (see chapter IX).

3. **Cortisone and TSH**

There is much evidence to show that cortisone is capable of inhibiting thyroid function (see point 7) and some evidence suggests that this may be effected *via* inhibition of TSH release in the pituitary.[54] Recently, direct evidence was brought forth to support this contention: Bakke and Lawrence[55] measured the TSH content of 203 human pituitaries obtained at autopsy or surgery, and found that the pituitaries of subjects who had been treated with prednisone contained the lowest amount of TSH observed in the whole series.

4. **Cortisone and the Pituitary Gonadotrophins**

The data on the excretion of gonadotrophins in the urine following cortisone administration in experimental animals are conflicting; there are reports suggesting increased gonadotrophin excretion, others showing no effect. In man, little direct information is available. In Cushing's syndrome characterized by increased glucocorticoid activity, and in patients on prolonged cortisone therapy, amenorrhea or oligo-menorrhea is frequently observed suggesting inhibition of gonadotrophic activity. This may also in part be the mechanism of the amenorrhea seen in the Stein Leventhal syndrome which presumably is due to hyper-corticism: here, in spite of low estrogen levels—which would make for high FSH production—the urinary gonadotrophin excretion is low, and there are no symptoms of menopause.

5. **Cortisone and MSH**

Cortisone and hydrocortisone are highly effective in inhibiting the release of MSH from the pituitary gland. Blood and urinary levels of MSH are known to be high in *primary* Addison's disease. It is believed that this is due to release of increased amounts of pituitary MSH resulting from the absence of inhibitory glucocorticoids.[56] This leads to the characteristic pigmentation which decreases under cortisone therapy. Cortisone administration can prevent the onset of hyperpigmentation in the patient who had undergone bilateral adrenalectomy. On the other hand, there may be decreased pigmentation in *secondary* Addison's disease (due to hypopituitarism) in spite of the presence of adrenocortical deficiency, in view of the failure of the MSH-producing pituitary cells.

The urinary MSH excretion is at low levels in Cushing's syndrome presumably because of inhibition of MSH production by the excess corticoids. According to Sulman, this may be utilized to differentiate Cushing's syndrome (due to adrenal tumor or hyperplasia) from Cushing's disease (due to pituitary basophilism) since in the latter the MSH level may be high.[57]

6. **Cortisone and the Posterior Pituitary**

No data are available concernnig the effects of cortisone on the production or release of the posterior pituitary hormones. In its peripheral actions, cortisone may be synergistic with or antagonistic to the antidiuretic hormone of the posterior pituitary depending on the existing physiological state.[58]

7. **Cortisone and the Thyroid**

There is a large body of experimental data as well as clinical observations con-

cerning relations between the adrenal cortex and the thyroid but our understanding of this interdependence remains somewhat confused. The thyroid found at autopsy in Addison's disease or in the bilaterally adrenalectomized animal shows no striking changes in size or structure except for some lymphocytic and plasma cell infiltration. In is generally agreed that cortisone or prednisone in large doses inhibits thyroid function. Thus, low radio-iodine uptake, low serum PBI and low secretion rate of hormonally bound iodine are found in patients on prolonged treatment with cortisone and in patients in stress situations (in whom the blood 17-hydroxysteroid levels are known to be high).[54] The exact mode of this action of cortisone is not known: it could be due to inhibition of TSH release (as mentioned above), to a direct inhibitory effect on the thyroid or to a block of the mechanism by which TSH influences iodine uptake. It has been demonstrated that while TSH influences uptake of radio-iodine in the operated animals, th injection of cortisone together with TSH prevents restoration. This indicates that the inhibitory effect of cortisone is not due to interference with TSH production. Patients on prolonged cortisone therapy may show hypercholesterolemia and other laboratory signs of hypothyroidism.The term 'corticogenic hypothyroidism' has been coined to label this state, but the patients usually do not exhibit the clinical picture of hypothyroidism.[59] (On the other hand, thyroid function is usually normal in Cushing's syndrome; and hypothyroid patients receiving sub-optimal doses of thyroxine show elevation of BMR during cortisone administration with the radio-iodine uptake remaining unchanged. The elevation of BMR is ascribed to increased peripheral utilization of thyroxine in the tissues).[60]

Cortisone has been effectively used in the therapy of sub-acute thyroiditis and Hashimoto's struma lymphomatosa. This his probably nothing to do with the inhibitory effect of cortisone on thyroid function. In these two diseases of the thyroid an auto-immune mechanism is believed to be operating, and the beneficial effect of cortisone therapy may be due to the known capacity of cortisone to interfere with antigen-antibody reactions.[61]

8. **Cortisone and Insulin**

Cortisone and other 11-oxygenated cortico-steroids stimulate gluco-neogenesis from protein and fat and are antagonistic to insulin. The exact mechanism of these actions is not yet understood; it has been suggested that cortisone may inhibit protein synthesis, i.e. it has anti-anabolic rather than catabolic effect. Cortisone also appears to inhibit some phase of glucose utilization although the locus of the block is not known.

Administration of cortisone in large amounts to normal subjects for 5-10 days may produce fasting hyperglycemia, glycosuria, diminished glucose tolerance, marked insensitiveness to insulin, all completely reversible. (However, these effects are less intensive and less consistent than the same effects produced by ACTH). These manifestations of carbohydrate metabolism disturbance are observed in Cushing's disease (which is characterized by excess production of gluco-corticoids); overt diabetes is uncommon. In patients with coexisting Addison's disease and diabetes, cortisone administration causes increase in the intensity of the diabetes and increase in insulin requirement. In Addison's disease (with absence of gluco-corticoids) there is deficient contra-insulin activity resulting in low fasting blood sugar and less responsiveness to insulin-hypoglycemia. The diabetogenic action of cortisone has been used, in combination with oral glucose tolerance tests, to discover potential diabetics: when cortisone was administered simultaneously with the glucose, diabetic type blood sugar curves were found 12 times as often among relatives of diabetics than among control individuals.

Cortisone potentiates the action of glucagon (in rabbits) suggesting that the insulin resistance induced by excessive amounts of cortisone may be in part a result of this potentiating effect on the glucagon contained in the insulin preparations.[62]

9. **Cortisone and Epinephrine**

Cortisone administration depresses the output of nor-epinephrine (but not of epinephrine).[63] Several clinical reports mention that prolonged cortisone administration produced hyperpigmentation similar to that seen in patients receiving ACTH. This would be difficult to explain in view of the fact that cortisone is not known to have any marked direct effect on the melanocytes; moreover, cortisone *inhibits* MSH release from the pituitary. Lerner believes that the darkening effect may be due to a lowering of nor-epinephrine production resulting from the administration of cortisone (see chapter X).

10. **Cortisone and Androgens**

Asde of its effect on sex steroids *via* ACTH and gonadotrophins cortisone may be involved directly in the production of androgen because of its possible conversion into androgen. This was first suggested by the observation that cortisone administration is capable of maintaining spermatogenesis in the hypophysectomized adrenalectomized rat while it does not prevent atrophy of the testicular interstitial tissue. It was then found that patients after castration and adrenalectomy maintained on cortisone therapy, continue to excrete small amounts of 17-ketosteroids, and that this excretion falls to zero when cortisone is withdrawn[64]. This is explained by the assumption that some of the administered cortisone is converted to androgen. The conversion is believed to take place in the liver and the cortisone/androgen conversion ratio appears to be very low. Female patients on prolonged cortisone therapy not infrequently develop acne and hypertrichosis, which was ascribed to this conversion. However, the 17-ketosteroids excreted in increased amounts on cortisone administration are 11-oxygenated, 17-keto-steroids which are biologically not androgenic and, accordingly, the acne and hypertrichosis remain as yet unexplained. (In contrast, the increased urinary 17-keto-steroids following administration of ACTH contain besides 11-oxygenated 17-ketosteroids also 17-ketosteroids lacking oxygen at C_{11}, such as androsterone and etiocholanolone, which are biologically androgenic; this readily explains the acne and hypertrichosis developing in patients on prolonged ACTH therapy)[65].

It may be recalled at this point that in hyperadrenocorticism associated with abnormally high 17-keto-steroid excretion—such as congenital cortical hyperplasia — administration of cortisone has the opposite effect on the urinary 17-keto-steroid excretion, i.e., it causes a prompt and marked *decrease* with relief of the signs of masculinization. This is effected *via* inhibition of ACTH producton, and the resulting great decrease of 17-keto-steroid production is but little altered by the simultaneous minimal cortisone-androgen conversion.

Cortisone can antagonize the effect of testosterone on the seminal vesicles in the castrated rat.[66]

11. **Cortisone and Estrogens**—see chapter XII.

CORTISONE
inhibits release of ACTH
may inhibit release of TSH, FSH, MSH
may antagonize somatotrophic effects of GH
may antagonize Thyroid function directly
antagonist of Insulin
can inhibit release of Nor-Epinephrine from Adrenal Medulla
can antagonize certain effects of Testosterone
may be converted (in very small ratio) to Androgen

XII. ESTROGENS

1. **Estrogens and Gonadotrophins**

As expressed in the term pituitary-gonadal axis, a feed-back mechanism exists between estrogens and gonadotrophins, some of the main manifestations of which are as follows: a) After removal of the ovaries in experimental animals considerable hyperplasia of the anterior pituitary develops with marked increase in the production of FSH, while LH is diminished and later disappears entirely. b) In the menopause, there is an increase in gonadotrophin excretion, 5 to 10 times above the pre-climasteric normal levels. In castration as well as in the natural menopause the high FSH production is related to the low level of circulating estrogens (the adrenals continue to produce a minimal amount of estrogens). c) Estrogen produced endogenously in physiological amounts in normal women during the menstrual cycle inhibits FSH

release, stimulates LH and LTH release from the anterior pituitary as outlined in Chapter IV. d) In castrated young women, administration of estrogen in *small* doses increases urinary FSH as well as LH excretion.[19] Administered in large doses, estrogen inhibits release of all three gonadotrophins in normal, castrated or post-menopausal women as well as in men.[19] The estrogen-gonadotrophin relationship is the basis for using the 24 hour urinary gonadotrophin excretion for the differential diagnosis between primary (ovarian) and secondary (pituitary) hypo-ovarianism; in the former the gonadotrophins are abnormally high because the ovary produces no estrogen that normally would inhibit gonadotrophin production, while in the latter gonadotrophin excretion is low because the deficient pituitary is unable to maintain normal gonadotrophin production. e) The low circulating levels of estrogen at the end of pregnancy allow release of prolactin from the anterior pituitary inducing secretion of milk in the mammary glands. Estrogen administered in large amounts at this time is capable to prevent postpartum lactation. f) In the male, estrogen administration in large amounts causes profound atrophy of the seminiferous tubules of the testis with cessation of spermatogenesis; this is believed to be due to inhibition of FSH production in the pituitary. A similar mechanism is responsible for the development of testicular atrophy in patients with hepatic cirrhosis or hepatitis where decreased inactivation of estrogen by the diseased liver results in high circulating estrogen levels leading to inhibition of pituitary gonadotrophin production with consecutive testicular atrophy.

2. **Estrogen and the Growth Hormone**

The growth spurt at puberty depends in both sexes on the sex hormones, which indicates some sort of synergism between GH and estrogen as well as between GH and androgen. It has been suggested that the pubertal growth spurt in girls may be due to adrenal androgen rather than ovarian estrogen: the urinary 17-keto-steroids are increased at puberty in girls (as well as in boys) which could be a sign of increased adrenal androgen production.[68] Estrogen hastens the fusion of epiphyseal junctions more than androgen does, which may explain the fact that the ultimate average height of girls is lower than that of boys. This may be the reason why administration of large amounts of estrogen to excessively growing girls may result in slowing down and terminating the growth.

3. **Estrogens and ACTH**

Bilateral oophorectomy is followed by decrease in the size of the adrenals, and estrogen administered in moderate doses leads to enlargement of the adrenals and elevated 17-hydroxysteroids in the blood.[69] Adrenomegaly cannot be produced in the hypophysectomized animal by estrogen administration. All this indicates that estrogen is capable of stimulating ACTH production in the anterior pituitary[70].

Hydrocortisone (as well as aldosterone) excretion is substantially increased in pregnancy. Part of this increase may be due to depressed degradation of these steroids.[71]

4. **Estrogen, TSH and Thyroxine**

Prolonged administration of large amounts of estrogen to experimental animals depresses thyroid function as shown by histology of the thyroid. This is believed to be due to inhibition by estrogen of the release of TSH from the pituitary. However, the radio-iodine uptake in women does not vary throughout the menstrual cycle, and there are no constant changes in the serum protein-bound iodine (PBI) after bilateral oophorectomy[72]. Myxedema is about four times as common in women as in men, but it is not known what role estrogen plays in the development of that disease.

Pregnancy is frequently accompanied by thyroid hyperplasia with increase of radio-iodine uptake by the thyroid and increased serum PBI, but there are no symptoms of thyrotoxicosis and no increased BMR beyond that attributable to fetal needs. There is increased thyroxine-binding capacity of the alpha globulin of the serum (the thyroxine-binding protein); this increase begins early in pregnancy, proceeds throughout the preg-

nancy and gradually disappears after delivery, coinciding with the alterations in the levels of the serum PBI. That these changes may be due to high levels of circulating estrogens—known to be present in pregnancy—is suggested by the experience that large doses of estrogen administered to normal males or females induce increases in serum PBI and in thyroxine-binding capacity of the serum alpha-globulin comparable to those seen in normal pregnancy.[73] Furthermore, it was found that the erythrocyte uptake of tri-iodo^{131}-thyronine *in vitro* is strikingly decreased in pregnancy. (It is also decreased in hyperthyroidism, hepatitis, hepatic cirrhosis, extensive metastatic malignancy and during dicoumarol treatment). The factor responsible for these changes resides in the blood plasma[74]—not in the erythrocytes—and the decreased erythrocyte uptake may be an effect of the increased thyroxine binding capacity of the plasma proteins. The physiological and clinical significance of these findings is not yet clear.

5. **Estrogens and the Ovary**

Indirectly, *via* the gonadotrophin, activity of the anterior pituitary, estrogens can profoundly influence the structure and functions of the ovary (see point 1. in this chapter) and can potentiate the effect of FSH on the Graafian follicle (see point 2, chapter IV) but have no direct, independent effect on the ovary. Concerning interactions of estrogen and progesterone, see point 2, chapter XIII).

6. **Estrogens, the Testis and Androgens**

Estrogens have no *direct* effect on the structure and functions of the testis. Indirect effects of androgens on the testis *via* pituitary gonadotrophins have been pointed out earlier in this chapter.

Interactions of estrogens and androgens may be antagonistic or synergistic depending on the target organ or process on which they act: i.e., antagonistic in acting on certain targets, synergistic in acting on others. (See Table 1.)

It is important to keep in mind that the two hormones are synergistic in their effect on pituitary gonadotrophic activity (both inhibit it), but by virtue of this very action they can antagonize each other's peripheral effects when one of the hormones is administered in large amounts. The use of estrogens in the therapy of prostatic cancer will illustrate this point. Most observers believe that the beneficial effect of estrogen on early prostatic cancer—when the cancer cells are vitally dependent on androgen support—is not due to any neutralization of the endogenously produced androgen at the malignant cell level. Rather, the administered large amounts of estrogen inhibit pituitary gonadotrophin release which leads to cessation of testicular androgen secretion, thus depriving the malignant cell of androgen support[75]. Analogous interpretation is given by some of the therapeutic effects of androgen in premenopausal breast cancer[75]. In other estrogen-androgen interactions the "battle of the hormones" takes place at the target level. In Table 1, on targets #3, 4, 5, 6, 7, 8, 12, 13, 14, 15, the sex hormones probably act at the target level, while on targets #1, 2, 9, 10, 11 the effects are mediated through the pituitary.

7. **Estrogens, the Adrenal Cortex and Corticoids**

Natural estrogens have no *direct* effect on the adrenal cortex as shown in experiments in which the ACTH-stimulated corticoid secretion of adrenal cortex slices in vitro was assayed.[77] (Some synthetic estrogens such as stilbestrol and ethinyl-estradiol strongly inhibited corticoid secretion in these experiments.) Indirect effect of estrogens on the adrenal cortex *via* ACTH of the pituitary was discussed in point 3 of this chapter.

Administration of estrogen raises the cortisone requirement in Addison's disease,[78] and the effect of estrogen on the uterus is lessened by cortisone,[78] indicating antagonism between the two circulating hormones. With feminizing tumors of the adrenal cortex the patients show excess production of adrenal estrogens as well as glucocorticoids, but the feminizing effects of estrogen are not abolished by the excess corticoids.

ESTROGENS
(in small amounts) may stimulate release of FSH and LH

	TARGET	EFFECT OF ANDROGEN	EFFECT OF ESTROGEN
I.	1. * Cancer of prostate	+	–
	2. * Cancer of breast	–	+
	3. Secretion of prostatic fluid[94]	+	–
	4. Sebaceous glands[95]	+	–
	5. Seminal vesicles[98]	+	–
	6. Vaginal epithelium[98]	–	+
	7. Thyroxine binding capacity of plasma proteins[73]	–	+
	8. Ratio $\frac{\alpha - \text{lipoproteins}}{\beta - \text{lipoproteins}}$ in plasma[96]	–	+
II.	9. * Release of pituitary FSH	–	–
	10. * Seminiferous tubules and spermatogenesis	–	–
	11. * Postpartum lactation	–	–
	12. Formation of bone matrix[97]	+	+
	13. * Serum inorganic phosphate level	–	–
	14. * Fusion of epiphyseal junctions	+	+
	15. * Pubertal growth spurt	+	+

TABLE 1. Some interactions between androgens and estrogens.

\+ stands for stimulation or increase; – for inhibition or decrease

* see text.

It is seen that in the actions listed under I. the two hormones appear as antagonists while in those listed under II. they appear as synergists.

(in large amounts) inhibit release of FSH and LH
may stimulate release of ACTH
may be synergistic with GH in growth at puberty
synergistic in certain actions with Progesterone
may depress Thyroid function
increase Thyroxine binding capacity of plasma proteins
may be antagonistic to Cortisone
may be synergistic with or antagonistic to Androgens

XIII. PROGESTERONE.

Progesterone is the secretory product of the luteinized granulosa cells of the corpus luteum. It is also produced by the placenta during the last two trimesters of pregnancy. It is probably constantly synthesized in the adrenal cortex of both sexes where it appears to be a key intermediary of corticosteroids. Its more important interrelations are with the pituitary gonadotrophins, with estrogens and the adrenals.

1. Progesterone and Gonadotrophins

Acting synergistically with estrogen, progesterone in small amounts can stimulate release of LH. Administered in large doses, it depresses in men as well as in women the release of FSH and LH, which is interpreted as another feedback regulatory mechanism.

2. Progesterone, the Ovaries and Estrogens

Mediating through the anterior pituitary gonadotrophins, progesterone may have both a stimulating and inhibitory effect on the ovaries—as indicated above—but is not known to have any *direct* effect on the ovaries. Circulating progesterone and estrogen are synergistic in some of their actions, but may be antagonists in other actions or without any mutual influence;[79] also, there are species differences. Thus, in their effects on the release of pituitary gonadotrophins, on the endometrium and the mammary tissue the two hormones are generally synergistic. They are also synergistic in their effect on the response of uterine muscle to oxytocin in the cat; both sensitize the cat uterus to oxytocin. However, in the rabbit and some other mammals progesterone renders the myometrium unresponsive to oxytocin while estrogen increases its sensitvity; and in the monkey and man neither hormone has any marked effect on the oxytocin sensitivity[80]. Progesterone is antagonistic to estrogen in so far as the latter's effect on the testis is concerned: estrogen administration in large amounts causes atrophy of the seminiferous tubules, but when progesterone is simultaneously given, atrophy and aspermatogenesis are prevented.

3. Progesterone and the Adrenals.

Progesterone administered in large amounts produces considerable atrophy of the adrenal cortex[81]; it is not known whether this is by direct effect or *via* inhibition of ACTH release from the pituitary.

Progesterone administered in physiological doses enhances the urinary excretion of sodium and chloride in normal men and women, presumably at the level of the renal tubules, and is in this sense an antagonist of aldosterone. This interaction which is not materially influenced by simultaneous estrogen administration may have a major role in the sodium metabolism of pregnancy and during the luteal phase of the menstrual cycle[82]. (This is in contradiction to earlier reports mainly in animals according to which progesterone administration is usually followed by salt and water retention.)

PROGESTERONE
(in small amounts) may stimulate release of LH
(in large amounts) inhibits release of LH and FSH
may be synergistic with or antagonistic to Estrogen
may suppress Adrenocortical function
may be antagonistic to Aldosterone

XIV. ANDROGENS

1. Androgens and Gonadotrophins

As expressed in the term pituitary-gonadal axis, there is a reciprocal relationship between gonadal androgens and the release of gonadotrophins of the pituitary. Castration in the male animal with its resultant absence of circulating androgen is followed by enlargement of the anterior pituitary, appearance of enlarged basophilic cells (castration cells) in the anterior lobe and greatly increased excretion of urinary gonadotrophins, both FSH and LH. These changes can

be inhibited by administration of androgen (or estrogen); however, androgen must be administered in much larger doses than estrogen for this purpose. This has led to the assumption that the epithelium of the seminiferous tubules of the testis normally produces an inhibitory hormone, either estrogen or a specific hormone, "inhibin." (Estrogen is present in the normal seminal discharge and in certain testicular tumors, and the stallion normally produces large amounts of an estrogen, equilenin.) Another possible cause of the increase of urinary gonadotrophins following castration is lack of utilization by the absent testicles rather than increased release of gonadotrophins.

Increased amounts of urinary gonadotrophins are a constant feature of the Klinefelter syndrome characterized by very small testes which on histological study show partial to complete hyalinization of the tubules with loss of spermatogenesis; the Leydig cells are quite prominent and often give the appearance of being increased in number. The majority of cases show normal accessory sexual organs except for gynecomastia. It has been postulated that the increased urinary gonadotrophins in this syndrome might depend upon a difference between the threshold of response of the anterior pituitary and the response of the accessory sex organs, the former being more sensitive than the latter. If this is so, a slight decrease in the amount of circulating testicular androgen could provoke increased gonadotrophic activity in the anterior pituitary without causing failure of the accessory sex organs. Neither this concept nor the "inhibin" theory has been conclusively proven, and it remains reasonable to assume that the rise of gonadotrophins might be merely the result of inability of the atrophic seminiferous tubules to utilize — and thereby inactivate — the gonadotrophins. The recent discovery that most patents with this syndrome are genetic females has no bearing on the mechanism of gonadotrophin increase since such increase occurs also in those patients who are genetic males.

Androgen, which is a weak inhibitor of pituitary prolactin production, is used in the prevention of postpartum lactation (in combination with estrogen which is a much more powerful inhibitor of prolactin production, see Chapter XII, only in order to counteract in those patients some undesirable side effects of the estrogen, such as endometrial proliferation, retardation of involution of the uterus, secondary breast engorgement and uterine bleeding[67].

2. Androgens and the Growth Hormone

There is some evidence that androgens may inhibit the release of growth hormone in the pituitary; on the other hand, circulating androgens may act synergistically with circulating growth hormone. Kinsell and his associates[83] have shown that androgenic steroids (and to a lesser extent estrogens as well) depress the high phosphate level and growth hormone level in the blood of acromegalics. High inorganic phosphate levels in the blood in prepubertal children are believed to indicate high growth hormone activity, and Hamilton[84] has shown that at puberty, in both sexes, as a result of the appearance of sex steroids, the blood inorganic phosphates decrease and remain at the decreased levels all during maturity.

The growth spurt that occurs at puberty depends in the male on androgen indicating synergism between GH and androgen at that age. The pituitary dwarf does not show increased rate of growth at the age of puberty but responds with some extra growth to administration of androgen. Children with adrenogenitalism or congenital adrenal hyperplasia show excessive transitory somatic growth. These observations have led to the administration of androgens in the therapy of deficient growth in boys. The synergistic action of GH and androgen may be at least partly explained by the fact that both are protein anabolic in the growing child.

3. Androgen and ACTH

Since ACTH presumably stimulates the adrenal cortex to secrete androgen, it may be expected that a feed-back mechanism exists between androgen and ACTH production. This concept is well supported by experiments in the rat:

Castration of the male rat is followed by adreno-cortical hypertrophy (especially in the zona fasciculata and reticularis) and this hypertrophy may be inhibited by androgen administration. Furthermore, androgen administered to normal male or female rats can cause adreno-cortical atrophy. In man, the available data are somewhat contradictory. Albright[85] found androgens to be of some value in correcting many of the manifestations of Cushing's syndrome, but the question whether these effects are mediated *via* ACTH production or due to direct action of the androgen on the adrenal cortex is difficult to decide. Bartter and associates[86] reported that testosterone administered to female patients with Cushing's syndrome caused decreased urinary 17-hydroxysteroid excretion. This they ascribed to decreased ACTH output because when androgen was administered together with ACTH, the effects of the exogenous ACTH on the urinary steroid excretion were normal, indicating that the androgen had no effect on the *action* of ACTH. Similar results were reported by Brooks and Prunty[87] after administration of 17-ethyl, 19-nortestosterone. Brown and Migeon[88] had administered large doses of testosterone to normal subjects; there was marked temporary lowering of plasma 17-hydroxysteroids, while simultaneous administration of ACTH had its normal effect of increasing the plasma 17-hydroxysteroids. These results indicate, although do not prove, that androgens can inhibit ACTH release in the pituitary. On the other hand, Gemzell and Notter[89] found no change in plasma 17-hydroxysteroids when large amounts of testosterone were given to normal subjects.

4. Androgens, TSH and Thyroxine

In man, administration of testosterone leads to reduction of radio-iodine uptake by the thyroid and lowering of plasma PBI to hypothyroid levels without clinical evidence of hypothyroidism[90]. The reduction of radio-iodine uptake could result from various processes, but suppression of TSH release from the anterior pituitary seems to be the most likely mechanism. The reduction of PBI levels under the effect of androgen has been attributed to another mechanism: reduction of the thyroxine binding capacity of the plasma proteins[90]. (This is the opposite of the effect of estrogens on the thyroxine binding capacity of plasma proteins. (See chapter XII.)

5. Androgens and the Adrenal Glands

That androgens can act on the adrenals *directly* (*i.e.*, independently from the pituitary) has been suggested by experiments in which cortisone-induced atrophy of the adrenal cortex in the rat was completely prevented by administration of testosterone and related steroids, and by the experience that combined cortisone-testosterone treatment can maintain normal adrenal size in the rat after hyphophysectomy[91]. This indicates that androgens have a stimulating effect on the adrenal cortex.

On the other hand, in man, testosterone given in large doses for several days before major surgical operations can prevent the usual post-operative rise of plasma 17-hydroxysteroids[89]; also, in adrenalectomized patients maintained on cortisone, testosterone caused decrease in the urinary excretion of steroid metabolites which originate from the exogenous cortisone, and precipitated an Addisonian crisis in one of the patients[89]. These changes were interpreted to indicate some kind of antagonistic influence of androgens on the adrenal cortex. However, Edwards and his associates[92] found that the excess urinary 17-hydroxysteroid excretion following partial gastrectomy was not influenced by intramuscular testosterone propionate administration; and when the direct effect of steroid compounds on corticoid secretion *in vitro* was studied it was found that testosterone had no effect on the ACTH stimulated adrenocortical slices. Further studies will be necessary to clarify the role of androgens in the control of the adrenal cortex and its steroids.

6. Androgens and the Testis

It is known that complete spermatogenesis depends not only on stimulation of the germinal epithelium of the testicular tubule by FSH of the pituitary but also on the presence of androgenic sub-

stances; and ICSH is needed for complete spermatogenesis only by virtue of its ability to stimulate production of androgen. That there is a *direct* stimulatory effect of androgen on the seminiferous tubule has been clearly shown in the hypophysectomized rat in which testicular atrophy and aspermatogenesis can be prevented by testosterone given immediately following hypophysectomy, and in which injection or implantation of testosterone in the tunica albuginea can maintain spermatogenesis in the tubules adjacent to the injected or implanted area[93]. Thus, androgen can damage the testes *indirectly* by inhibiting FSH and ICSH release, and stimulate the seminiferous tubules by *direct* action.

In man, testosterone administration tends to decrease temporarily sperm count and fertility but rarely leads to severe testicular atrophy. That this effect of androgen is mediated through the pituitary *via* inhibition of gonadotrophin release is indicated by the observation that no decrease in sperm count occurs when pituitary gonadotrophin is given concurrently with androgen. In man, too, androgen can stimulate spermatogenesis by direct action on the testis as indicated by reports showing that in several cases of human hypopituitarism sperm production occurred under treatment with testosterone without rise in urinary gonadotrophin.

7. Androgens and the Ovary

Androgen circulating in excess can suppress estrogen production by the ovary. This is the reason for using testosterone in the treatment of metastatic breast cancer in pre-menopausal women[75]; there may be additional usefulness of this therapy by virtue of the anabolic action of testosterone on bone formation. Whether androgen administration suppresses estrogen production by inhibiting pituitary gonadotrophic activity or by direct inhibition of the ovaries or by antagonistic action on the circulating estrogen, is not known.

Androgen can damage the ovary in the fetus. In the cattle, male and female twin fetuses sometimes have common placental supply and, under the influence of androgen produced by the male fetus, the ovary of the female twin assumes ambisexual characteristics ('freemartin'). Some degree of ambisexuality or pseudohermaphrodism is also seen in female babies—animal as well as human—when androgen (or progesterone) had been administered in large amounts to the mother during pregnancy. Perhaps the small —polycystic ovaries in the Stein-Leventhal syndrome result from damage produced by excess adrenal androgens in these patients.

Conversion of androgens (19-carbon steroids) to estrogens (18-carbon steroids) may be listed among interactions of these two hormones. There is evidence that at a very small ratio such conversion may occur. Thus, it has been demonstrated that in castrated or postmenopausal women injections of testosterone result in increase of urinary estrogen. Such result has also been reported in normal and eunuchoid men.[76]

ANDROGENS
inhibit release of FSH and LH
may inhibit release of GH, ACTH, TSH
are synergistic with GH in growth at puberty
reduce Thyroxine binding capacity of plasma proteins
may stimulate Adrenal Cortex directly
stimulate the seminiferous tubules of the Testis directly
may be synergistic with or antagonistic to Estrogen
may be converted (in small ratio) into Estrogen

COMMENT

An attempt has been made to review briefly the most important hormonal interrelations with emphasis on their role in the diagnosis and therapy of endocrine disturbances. Efforts were made to analyze some interactions as to locus and mechanism as such knowledge greatly facilitates the understanding of these complex phenomena.

REFERENCES

1. Beck, J. C., et al.: Annals Int. Med., *49*:1090, 1958.
2. D'Angelo, S., et al.: J. Clin. Endocr. & Metab., *11*:1237, 1951.
3. Willebrand, A. F., et al.: Diabetes, 7:119, 1958.
4. Foa, P. P., et al.: Recent Progr. in Horm. Res., Acad. Press, *XIII*:473, 1957.
5. Young, J. G.: J. Clin. Endocr. & Metab., *11*: 531, 1951.
6. Beck, J. C., et al.: Annals Int. Med., *49*:1090, 1958.

7. Rawson, B. W.: Ciba Found. Coll. on Endocr., The Blakiston Co., *IV*:294, 1952.
8. Parkes, A. S.: Physiol. Rev., *25*:203, 1945.
9. Moore, C. R.: J. Clin. Endocr. & Metab., *13*: 330, 1953.
10. Vanderlaan, W. P.: J. Clin. Invest., *32*:609, 1953.
11. Sandberg, H., et al.: J. Clin. Endocr. & Metab., *18*:1268, 1958.
12. Muller, A. F., et al.: Lancet, *2*:1021, 1956.
13. Heard, R. D. H.: Rec. Prog. Horm. Res., *XII*: 73, 1956.
14. Moore, F. D.: Rec. Prog. Horm. Res., *XIII*:511, 1957.
15. Larzlere, R. G., et al.: Arch. Int. Med., *99*:888, 1957.
16. Soffer, L. J., et al.: J. Clin. Endocr. & Metab., *10*:594, 1950.
17. Christy, N. P., et al.: J. Clin. Invest., *34*:899, 1955.
18. Leonard, S. L.: Anat. Rec., *57*:45, 1933.
19. Albert, A.: Rec. Prog. Horm. Res., *XII*:227, 1956.
20. Hibbitt, L. L., et al.: J. Clin. Endocr. & Metab., *18*:1315, 1958.
21. Lorincz, A. L.: in Rothman's Physiol. & Biochem. of the Skin, U. of Chicago Press, p. 544.
22. Shizume, K. and Lerner, A. B.: J. Clin. Endocr. & Meab., *14*:1491, 1954.
23. Nagareda, C. S. and Gaunt, R.: Endocr., *48*: 560, 1951.
24. Beck, J. C., et al.: Science, *125*:884, 1957.
25. Gaunt, R. and Birnie, J. H.: Horm. and Body Water, Amer. Lecture Series #103. Thomas, Publisher, Springfield. 1951.
26. Robinson, K. W., et al.: J. Clin. Endocr. & Metab., *17*:320, 1957.
27. Robbins, J. and Rall, J. E.: Rec. Progr. Horm. Res., *XIII*:161, 1957.
28. Goldberg, R. C., et al.: Endocr., *56*:560, 1955.
29. Castor, C. W. and Beierwaltes, W.: J. Clin. Endocr. & Metab., *16*:1026, 1956.
30. Perlmutter, M. and Slater, S.: J. Amer. Med. Assoc., *158*:718, 1955.
    Johnson, D. E., et al.: J. Clin. Endocr. & Metab., *19*:317, 1959.
31. Halmi, N. S. and Bogdanove, E. M.: Proc. Soc. Exp. Biol. Med., *77*:518, 1951.
32. Cortell, R. and Rawson, R. W.: Endocr., *35*: 488, 1944.
33. Williams, G. A., et al.: J. Clin. Endocr. & Metab., *17*:347, 1957.
34. Mikulaj, L. and Nemeth, S.: J. Clin. Endocr. & Metab., *18*:539, 1958.
35. Leifer, E. and Hollander, W.: J. Clin. Endocr. & Metab., *13*:1264, 1953.
36. Underdahl, L. O., et al.: J. Clin. Endoc. & Metab., *13*:20, 1953.
37. Soffer, L. J.: Dis. of the Endoc. Glands, p. 247. Lea & Febiger, Philadelphia, 1956.
38. Salter, J. M. and Best, C. H.: Brit. Med. J., *II*:353, 1953.
39. Elmadjian, F., et al.: J. Clin. Endocr. & Metab., *16*:876, 1956.
40. Long, C. N. H., et al.: Fed. Proc., *6*:416, 1947.
41. Vogt, M.: J. Physiol., *102*:341, 1943.
42. Rawson, R. W.: Rec. Progr. Horm. Res., *I*:99, 1947.
43. Friedgood, H. B. and Uotila, V. V.: Am. J. Physiol., *129*:724, 1940.
44. Sawyer, C. E. and Everett, C. E.: Endoc., *46*: 536, 1950.
45. Lerner, A. B. and Takashashi, Y.: Rec. Prog. Horm. Res., *XII*:303, 1956.
46. Ginsburg, J. and Paton, A.: Lancet, *2*:491, 1956.
47. Groen, J., et al.: Diabetes, *7*:276, 1958.
48. Bongiovanni, A. M. and Eberlein, W. R.: Pediatrics, *6*:628, 1955.
49. Perloff, W. H., et al.: J. Am. Med. Assoc., *167*: 2041, 1958.
    Gold, J. J. and Frank, R.: Am. J. Obst. Gyn., *75*:1034, 1958.
50. Farrell, G. L., et al.: Endocr., *58*:104, 1956.
51. Summerskill, W. H. J. and Crabbe, J.: Lancet, *2*:1091, 1957.
52. Marx, W., et al.: Endocr., *33*:102, 1943.
53. Kramar, J., et al.: Endocr., *60*:589, 1957.
54. Sherer, M. G. and Siefering, B. B. N.: J. Clin. Endoc. & Metab., *16*:643, 1956.
55. Bakke, M. D. and Lawrence, N. L.: J. Clin. Endoc. & Metab., *19*:35, 1959.
56. Lerner, A. B., et al.: J. Clin. Endoc. & Metab., *14*:1463, 1954.
57. Sulman, F. G.: J. Clin. Endoc. & Metab., *16*: 775, 1956.
58. Thorn, G. W., et al.: New Eng. J. Med., *284*: 284, 1953.
59. Rawson, R. W.: Proc. 2nd Clin. ACTH Confer., *2*:95, 1952.
60. Hill, S. R., et al.: J. Clin. Endoc. & Metab., *10*:1375, 1950.
61. Doniach, D. and Roitt, I. M.: J. Clin. Endocr. & Metab., *17*:1293, 1957.
62. Lazarus, S. S., et al.: J. Clin. Endoc. & Metab., *17*:542, 1957.
63. Luft, E. and von Euler, U. S.: Metabolism, *1*: 179, 1952.
64. Engel, L. L., et al.: Proc. Am. A. Cancer Res., *1*:14, 1953.
65. ieberman, S., et al.: Fed. Proc., *9*:196, 1950.
66. Pincus, G. and Dorfman, R. T.: Fed. Proc., *14*:15, 1955.
67. Presto, B. L. and Caypinar, E. Y.: J. Am. Med. Assoc., *169*:130, 1959.
68. Wilkins, L.: Endoc. Dis. in Childhood, p. 16. Thomas, Springfield, 1957.
69. Wallace, E., et al.: Proc. Soc. Exp. Biol. Med., *95*:805, 1957.
70. Ellison, E. T. and Burch, J. D.: Endocr., *20*:746, 1936.
71. August, J. T., et al.: New Eng. J. Med., *259*: 917, 1958.
72. Stoddard, F. J., et al.: J. Clin. Endoc. & Metab., *17*:561, 1957.
73. Dowling, J. T.: J. Clin. Endoc. & Metab., *16*: 280 and 1491, 1956.
74. Hamolski, M. W., et al.: J. Clin. Endoc. & Metab., *17*:33, 1957.
75. Nathanson, I. T.: Glandular Physiol. & Ther., p. 463. Lippincott Co., 1954.
76. Callow, R. K.: Proc. Roy. Soc. Med., *31*:841, 1938.
77. McKerns, K.: Endocr., *60*:130, 1957.
78. Sellye, H.: Textbook of Endocr., p. 64. Montreal, 1948.
79. Courier, R.: Vitamins & Hormones, *VIII*:179. Acad. Press, N. Y., 1950.
80. Shelesnyak, M. C.: Rec. Prog. Horm. Res., *XIII*:318, 1957.

81. Clause, H. J.: Endocr., *27*:989, 1940.
82. Landau, R. L. and Lugibihl, A. B.: J. Clin. Endoc. & Metab., *18*:1237, 1958.
83. Kinsell, L. W., et al.: J. Clin. Endoc. & Metab., *8*:1013, 1948.
84. Hamilton, J. B. and Bunch, L. D.: J. Clin. Endoc. & Metab., *18*, 1958.
85. Albright, F.: Harvey Lect., *38*:123, 1943. The Science Press, Lancaster, Pa.
86. Bartter, F. C., et al.: J. Clin. Endoc. & Metab., *9*:663, 1949.
87. Brooks, R. W. and Prunty, F. T.: J. Endocr., *15*:385, 1957.
88. Brown, H. and Migeon, C.: J. Clin. Endoc. & Metab., *16*:1227, 1956.
89. Gemzell, C. A. and Notter, G.: J. Clin. Endoc. Metab., *16*:483, 1956.
90. Keitel, H. K. and Sherer, M. G.: J. Clin. Endoc. & Metab., *17*:855, 1957.
91. Zizine, L. A., et al.: Endocr., *47*:97, 1950.
92. Edwards, K. M., et al.: J. Clin. Endoc. & Metab., *17*:1460, 1957.
93. Dvoskin, S.: Anat. Rec., *99*:329, 1947.
94. Huggins, C.: Harvey Lect., *42*:148, 1947.
95. Rony, H. R. and Zakon, S. J.: Arch. Derm. & Syph., *48*:601, 1943; and *52*:323, 1945.
96. Furman, R. H. and Howard, R. P.: Ann. Int. Med., *47*:975, 1957.
97. Albright, F. and Reifenstein, E. C., Jr.: Parathyroid Glands and Metabolic Bone Disease. Williams & Williams, 1948.
98. Luft, R. and Olivecrona, H.: J. Neurosurg., *10*:301, 1953.
Dingman, J. F., et al.: New Eng. J. Med., *260*: 977, 1959.
99. Fajans, S. S.: J. Clin. Endoc. & Metab., *18*: 271, 1958.
Gurling, K. J., et al.: J. Clin. Endoc. & Metab., *19*:621, 1959.